INACTIVATION OF *CRYPTOSPORIDIUM PARVUM* IN NATURAL WATERS USING FREE CHLORINE

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Section 1
Introduction

1.1 Introduction and Perspective

Waterborne transmission of disease has been documented throughout history. One of the greatest contributions to 20th century society has been the advent and application of water treatment technologies. However, analysis of current World Health Organization (WHO) estimates of illness and mortality and epidemiological investigations in the United States still indicates that the threat to public health from waterborne disease still exists.

There are a plethora of contaminants (both chemical and microbiological) that impose a direct risk to public health in the United States. The United States Environmental Protection Agency (EPA) reviews these contaminants and focuses its efforts upon the chemicals and microorganisms that are entered on the Contaminant Candidate List (CCL). Although it has never appeared on the CCL, a large amount of research during the 1990s was focused upon *Cryptosporidium parvum*, a pathogenic waterborne parasite, to find methods and techniques to measure, remove, and inactivate it in drinking water supplies. *C. parvum* gained national public attention in 1993 when cryptosporidiosis in Milwaukee, Wisconsin infected an estimated 403,000 people and killed an estimated 65 to 100 people (MacKenzie, et al., 1994). The following year, an outbreak occurred in Las Vegas, Nevada. In this outbreak, 78 people contracted cryptosporidiosis resulting in 41 deaths within 12 months.

"The cryptosporidiosis outbreak in Las Vegas is unique in that tap water is indicated as the source of the problem; yet, the water treatment process, the distribution system and the quality of the water produced meets, in fact exceeds, acceptable sanitary standards" (Roefer, et al., 1996).

The Las Vegas outbreak (53% morbidity rate) clearly portrays the public health challenge associated with optimizing water treatment techniques and protecting public health, especially amongst sensitive populations.

Treatment strategies to protect treated water supplies, particularly during storage and distribution, have included the introduction of disinfectants such as chlorine. Chlorine disinfection practices have been under great scrutiny concerning the production of suspected carcinogenic byproducts and the true efficacy against *C. parvum*. The truth to these concerns

has tremendous implications within the drinking water industry in terms of economic, regulatory, and social costs.

1.2 Review of Published Research

There have been numerous *C. parvum* studies focused on detection, analysis, environmental distribution, and treatment techniques. The treatment techniques have primarily focused on removal techniques by filtration and inactivation using a wide range of disinfection technologies. The inactivation studies have focused on CT (Concentration-contact Time) conditions. Ozone has been reported to achieve the highest levels of inactivation compared to other chemical disinfectants (Korich, et al., 1990 and Finch, et al., 1997). Chlorine, the most commonly used disinfectant in the United States, has been determined to be ineffective in attaining acceptable inactivation at CT values typical of municipal water utilities (Driedger, et al., 2000; Gyurek, et al., 1997; Finch, et al., 1997; Fayer, 1995; and Korich, et al., 1990). All these studies have been conducted in batch systems with disinfectant demand-free environments. These systems are uncharacteristic of natural waters treated at municipal facilities and of the flow-through systems used in these facilities. Furthermore, the estimate of the rate constant for *Cryptosporidium* inactivation depends on the chlorine decay model if natural waters are used in batch systems.

1.3 Research Goals and General Approach

This study incorporated several unique techniques to examine the effect of disinfectant demanding substances in a flow-through system. The experimental design has incorporated the evaluation of two different waters: a natural surface water (Lake Mendota, Wisconsin) and a conventionally treated (coagulation, flocculation, sedimentation, and filtration) water. A completely mixed flow-through reactor (CMFTR) scheme was used to simplify some of the mathematical modeling and to make the *Cryptosporidium* inactivation constant independent of the chlorine decay model. The system was operated as several CMFTRs in parallel to evaluate the same water sample for a range of disinfectant concentrations and nominal detention times within the reactors. Enumeration and viability analyses were conducted using flow cytometric cell sorting (FCCS) under the supervision of

a highly trained and experienced analyst. The goal of the study was to validate the conclusions of previous chlorine inactivation studies, generate mathematical models to predict the effects of chlorine inactivation, and propose inactivation CT values.

Currey (2001) proposed several recommendations for future work based upon her analysis of the research. Her recommendations focused upon reactor conditions, chlorine decay kinetics, and disinfection kinetics. Specifically, she proposed the following:

- Reactor Conditions:
  - Conduct a tracer study to determine if mixing conditions can be improved by increasing the size of the stir bar.
  - Ensure all experiment steady-state is not achieved until 3.6 times the nominal detention time to achieve t_{95} values.
- Chlorine Decay Kinetics:
  - Evaluate disinfectant decay at smaller detention times to describe the initial decay of the disinfectant.
- Disinfection Kinetics:
  - Continue to conduct inactivatin experiments at increased CT conditions.
  - Target inactivation experiments to maintain a steady-state pH of 6.0.

Section 2
Cryptosporidium parvum

Cryptosporidiosis was once considered a rare disease, but data since the 1980s now show that "cryptosporidia are not rare opportunistic pathogens but rather are the cause of common, worldwide infections in healthy children and adults (Goodgame, 1996)."

2.1 Properties

2.1.1 Physical Characteristics

Cryptosporidium parvum (*C. parvum*) is an obligate, intracellular, coccidian parasite transmitted by ingestion of food and water contaminated with infected *bovine* feces. The naming of this parasite is derived from the Greek *crypto*, meaning hidden spore.

C. parvum is present in an environmentally resistant, spherical oocyst approximately 4 to 6 μm in diameter. This oocyst contains four sporozoites and has a structural "seam" that is commonly referred to as the "suture." This oocyst can survive up to 18 months in favorable conditions (moisture and shade) and is resistant to most disinfectants (Fayer, 1997). The important physical characteristics of the oocyst are the presence of two cell walls that protect the infectious sporozoite and a structural anomaly in the oocyst's construction (the suture). *Cryptosporidia* is closely related to other parasites of public health concern, namely *Isospora belli* and *Toxoplasma gondii*. It has a smaller diameter when compared to the oocysts of *Isospora belli* (20 to 30 μm) and *Toxoplasma gondii* (8 to 12 μm) and it also has one fewer barrier than these protozoa, as highlighted in Figure 2a. When compared to bacterial cells, the *C. parvum* oocyst is somewhat larger and has one more barrier than these microorganisms.

2.1.2 Taxonomy

Cryptosporidium parvum is the only member of the genus *Cryptosporidium* known to be infectious to humans (Goodgame, 1996). The number of species reported varies throughout the literature. Fayer and Ungar (1986) first reported twenty species; named according to the host. In addition to these twenty species, the CDC (1997) specified two species to include *wrairi* (guinea pig) and *serpentis* (lizard and turtle). In contrast, *Cryptosporidium* was

reported by others to be limited to four species (two found in mammals and two found in birds) by Rose (1988), Walker, et al. (1998), and Barer, et al. (1990). In yet another publication, Fayer, et al. (1997) listed eight different species (see Figure 2b). This variability in reporting the number of species has changed as different analytical tools evolved to assist in taxonomical classification. Nonetheless, it is agreed upon that *C. parvum* is the only species that is infectious to humans.
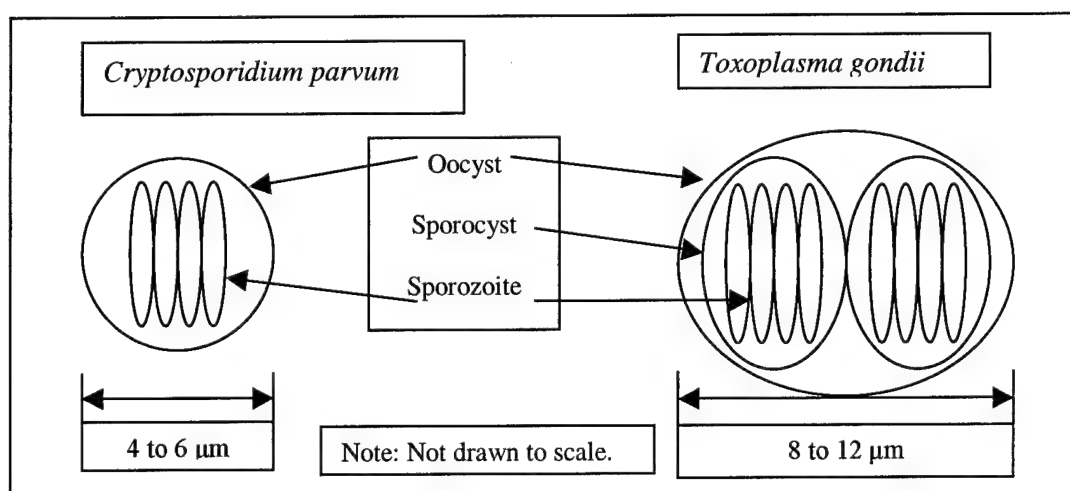
Figure 2a Size comparison and major cell structures of *C. parvum*.

Taxonomic Classification

- Kingdom: Protista
- Phylum: Apicomplexa (Sporozoa)
- Class: Sporozoea
- Subclass: Coccidia
- Order: Eucoccidiorida
- Suborder: Eimeriorina
- Family: Cryptosporidiidae
- Genus: Cryptosporidium
- Species: *baileyi, felis, meleagridis, muris, nasorum, serpentis, wrairi, parvum*
- Strains (parvum):   Type 1 (H)
                      Type 2 (C)

Figure 2b *Cryptosporidium parvum* taxonomical classification.

Recent studies have shown that *C. parvum* exists as no less than two distinct strains (Chappell, et al., 1999; Fayer, et al., 1997; and Peng, et al., 1997). This is reported as no less than two strains because some believe that two additional strains may exist. The impact of these strains upon humans has not been reported. At this time, Strain 1 or "H" (for human) is believed to be the only strain that is strictly infectious to humans. Strain 2 or "C" (for calf) is infectious to a range of animals and is transmitted between animals and humans.

2.1.3 Environmental Resistance

C. parvum is resistant to certain environmental factors and survival in the environment depends on the stage in the lifecycle and on the external conditions present. The oocysts, with both oocyst and sporozoite walls intact, are very resistant to external environmental conditions such as cold and water. Oocysts have demonstrated a lifespan of days to weeks in water, whereas the sporozoites only survive for minutes to hours in water. Oocysts have proven to retain viability and infectivity after "months in cold moist environments, such as lakes and streams, and even after freezing at $-15°C$ for 8 to 24 hours (Sterling and Marshall, 1999)." However, certain environmental factors that plague most parasitic organisms when they are outside their host create problems for *C. parvum* as well. "Oocysts can be rendered noninfectious when temperatures of $64.2°C$ or higher are held for 2 minutes and are susceptible to drying (desiccation) at 18 to $28°C$ for more than 4 hours (Sterling and Marshall, 1999)."

2.1.4 Lifecycle

As noted earlier, *C. parvum* exists as a thick-walled oocyst approximately 4 to 6 μm in diameter. Within the oocyst wall are four, infectious, crescent-shaped sporozoites. Upon ingestion and arrival within the small intestine, the oocyst wall will open along the suture and the sporozoites will be released. This process is known as excystation. The sporozoites will then attach to and invade the epithelial cells (internal lining) of the intestinal tract. Conditions for excystation usually require reducing conditions, however different conditions such as heat or external attack of the oocyst wall may induce the release of the sporozoites.

As defined, *Cryptosporidia* are obligate parasites. This means that they require a host to complete their lifecycle. They are further classified as monoxenous because the lifecycle is completed within one host. For *C. parvum*, the required host is a member of the *bovine* family, typically neonatal calves. It is here, within the intestinal tract of infected cattle that the reproduction lifecycle will occur. The lifecycle of *C. parvum* is illustrated in Figure 2c and detailed in Appendix A. Included in the cycle is both asexual and sexual reproduction.
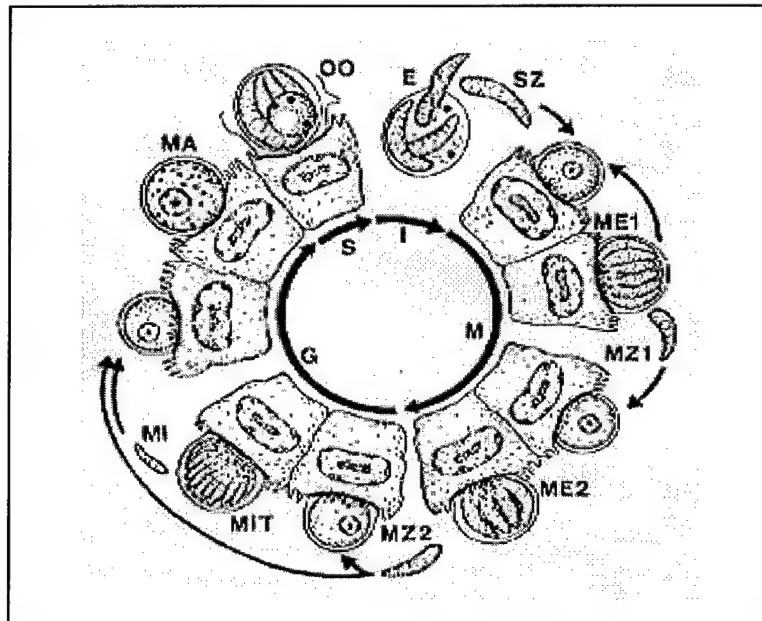
Figure 2c. *Cryptosporidium parvum* Lifecycle (abbreviations are defined in Appendix A). Source: http://www.ksu.edu/parasitology/basicbiology.html with permission

C. parvum has three common lifecycle characteristics shared by all pathogenic protozoa (Goodgame, 1996):

- The protozoa infect enterocytes (the epithelial-lining cells of the intestine). Invasion below the epithelial surface is not common.
- The infections are intracellular (within the enterocyte).
- The final product of maturation is the infectious particle, which is excreted into the intestine.

It is estimated that approximately 20 percent of the zygotes formed from the sexual cycle "develop into 'thin-walled oocysts', which are autoinfective lifecycle forms that can maintain the parasite lifecycle in the host (Sterling and Marshall, 1999 and Fayer, 1997)."

This lifecycle can develop and mature a new generation within 12 to 14 hours. This fast pace can encourage spreading of the infected areas because the ileum (beginning region of the intestine) is soon overcrowded and new host cells are sought. This is believed to be the key to the success of the parasite. The lag time (prepatent period) between infection and the first feces shed with oocysts is generally 4 to 7 days. Patency, which is the length of time oocysts are shed in the feces, generally lasts 6 to 12 days. "As few as 2 billion and as many as 20 billion oocysts can be collected during a single 24 hour period from calves during peak oocysts shedding (Fayer, 1997)."

2.1.5 Hosts and Vectors

Members of the *bovine* family, particularly neonatal calves are the only known host. Calves become infected with *C. parvum* by fecal-oral transmission and/or the intake of contaminated water or food.

It has been demonstrated that infection can result from the ingestion of water, fecal matter, and food. A carnivorous cycle seems an unlikely vector for human infectivity as no known "tissue cyst" form has been identified. A vector is defined as a carrier of the organism and may not become infected from the parasite. Environmental cycles can propagate within certain communities such as farms or specific wildlife environments as well as within certain human settings such as daycare facilities or swimming pools.

2.2 Environmental Occurrence

2.2.1 Historical Discovery

The history of *Cryptosporidium* dates back to the late 1890s when it was first discovered by Clark in 1895 (Fayer and Ungar, 1986). E. E. Tyzzer, an American parasitologist, was credited with describing the morphology and lifecycle in 1912. In 1971, it was first reported in association with bovine diarrhea and in 1976, two independent groups reported the first cases of human cryptosporidiosis. In 1984, the first waterborne outbreak associated with *Cryptosporidium* was documented.

2.2.2 Environmental Pathways

The fecal-oral route is the most common route of transmission. Thus, parents of young children and childcare workers are more susceptible to exposure via this route. Environmental occurrence is closely associated with contact to cattle fecal matter. Cases have documented infection directly related to the ingestion of contaminated water or food. Water not treated to remove oocysts is also a possible pathway for infection. The United States Centers for Disease Control and Prevention (CDC) lists drinking water and swimming pools as the top two sources of transmission. Zoonotic (animal-to-person) infections can result from contact with pets, farm animals, veterinary settings, and laboratory animals. There is no documented evidence to support airborne modes of contamination.

2.2.3 Geographical Distribution

"*Cryptosporidium* oocysts have been detected in wastewater, pristine surface water, surface water receiving agricultural runoff or contaminated by sewage, ground water under the direct influence of surface water, water for recreational use, and drinking water (EPA, 2000)." Distribution of the disease is worldwide and infection is more common in warmer climates and at lower altitudes (Fishback, 1992). "Recent studies indicate that *Cryptosporidium* oocysts are present in 65 to 97% of surface waters tested throughout the country" (Juranek, 1995). LeChevallier and Norton (1995) report *Cryptosporidium* was present in 60.2 percent of 347 surface water samples collected for a study conducted between 1988 and 1993 with an average concentration of 2.7 oocysts/liter (range: 0.07 to 484 oocysts/liter). Although these data indicate that *Cryptosporidium* are ubiquitous, it does not indicate viability or risk from waterborne pathogens.

"Serological surveys indicate that 80% of the population has had cryptosporidiosis (USFDA, 2000)." Seroepidemiologic surveys have documented a large percentage of human infectivity with the following prevalence rates in examined stool samples:

- 1 to 3% in Europe and North America
- 5 to 10% in Asia and Africa

Prevalence rate is defined as the number of disease cases in a certain population at a particular point in time. Despite low prevalence rate in stool sampling, 32 to 58% of the test

populations in developed countries contained antibodies to *Cryptosporidium* (Goodgame, 1996).

2.2.4 Occupational and Sensitive Populations

Any occupation that requires interaction with cattle (i.e. dairy farmers, zookeepers, or veterinarians) could be considered to have an increased risk. Occupations that tend to be exposed to fecal matter (i.e. hospital workers, day care workers) may also indicate higher percentages of infection based upon exposure to the fecal-oral route of transmission. However, occupational risks can be minimized with good personal hygiene practices and sanitary work conditions.

Sensitive populations include infants, pregnant women, elderly, and individuals with immunocompromised systems (HIV, organ transplant recipient, or patients undergoing chemotherapeutic treatment). Cryptosporidiosis is very common among AIDS infected individuals and is a leading candidate of opportunistic diseases that affect these individuals in the later stages of infection. Increases in cryptosporidiosis have been reported due to an increasing immunocompromised population, estimated in the U.S. at one-fifth of the population (CDC, 1997).

2.3 Health Effects

2.3.1 Routes of Entry and Incubation Time

Cryptosporidiosis is a medical condition that gained an enormous amount of publicity following the Milwaukee, Wisconsin outbreak in 1993. Cryptosporidiosis is an acute intestinal tract disease that is directly linked to human infection from the *Cryptosporidium parvum* species. Symptoms associated with infection include profuse and watery diarrhea along with abdominal pain, nausea, fever, malaise, and fatigue. However, it may be fatal to immunocompromised or immunosuppressed individuals. There is epidemiological evidence for protective immunity.

"Among cattle workers, repeated infections with *cryptosporidia* occur, but the second and third infections are much milder than the first. Similarly, in areas where cryptosporidiosis is common, permanent residents frequently acquire asymptomatic or clinically mild infections, but travelers become very sick (Goodgame, 1996)."

The most sensitive communities to *C. parvum* infection are those individuals diagnosed with the acquired immunodeficiency syndrome (AIDS). Incubation periods have been reported from "5 to 28 days with a mean of 7.2 days for human infection onset (Sterling and Marshall, 1999)." Currently, there is no prescribed medical intervention or definitive prevention available.

2.3.2 Target Cells, Tissues, and Organs

The epithelial cells of the small intestine are typically the most common cells targeted with infection, but all organs of the gastrointestinal tract may be affected. It is hypothesized that once invaded by the parasite; the epithelial cells will release cytokines (enzyme proteins that release an immune response). This results in the intestinal secretion of chloride and water, thus inhibiting absorption of water by the intestine (Goodgame, 1996).

2.3.3 Potency

How many oocysts are required to induce infection? Reported values for an ID_{50} were reported for both animal and human infectivity studies. An ID_{50} is the Infectious Dose that would result in 50% of the tested population testing positive for infection

2.3.3.1 Animal Infectivity Data

There are several studies that report an ID_{50} based upon mice models. Some of these reports provided no data and thus are not reported. Ernest, et al. (1986) estimated the ID_{50} in neonatal Swiss-Webster mice to be between 100 and 500 oocysts. Finch, et al. (1993) reported the ID_{50} to be 79 oocysts. Figure 2d summarizes the data presented in Finch's study. However, this is the lowest dose that causes 50% of the test population as increased doses caused a decrease and then subsequent increase in infection. There are several sources of variation in animal model systems, such as the strain of mouse used, the age of the mouse, the infectivity of the oocysts, and the strain of *C. parvum* (Finch, 1993). Animal infectivity data is open to controversy and criticism to using animals in inhumane manners and the "scaling-up" of data to be equivalent to human body mass/weight comparison.

C. parvum Oocyst Dose Responses in 4-day old CD-1 Mice
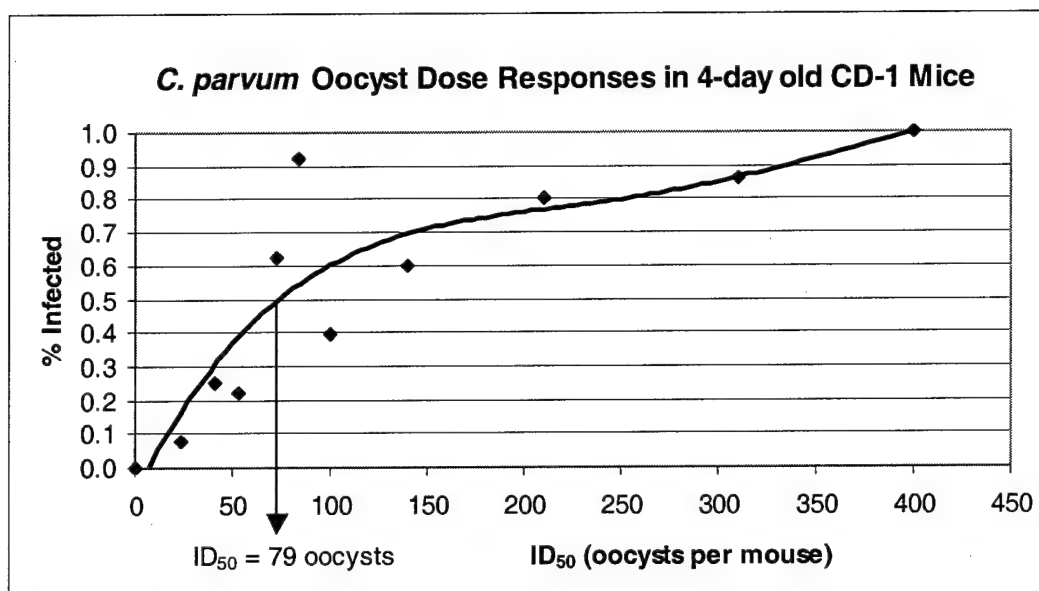
Note: Adapted from Finch, et al., 1993.

Figure 2d *C. parvum* Oocysts Doses and Responses in 4 Day Old CD-1 Mice

2.3.3.2 Human Data

Without doubt the most reliable method to obtain related infectious doses is with human studies. LeChevallier and Norton (1995) report the 10^{-4} annual risk is 3×10^{-5} oocysts/liter (based upon consumption of 2 L of water per day for a 70 kg person) for *Cryptosporidium.*

Okhuysen, et al., (1999) investigated the infectivity of three different isolates of the Type C genotype. Table 2.1 summarizes the three isolates, the ID_{50}, and the average duration of diarrhea. From this data, the TAMU isolate proved to be infectious at smaller doses (9 oocysts) and with a longer duration of diarrhea (94.5 hours) than the other two isolates. The definition of diarrhea was established as having greater than 3 stools in an eight-hour period or greater than 4 stools in 24 hours. The important difference regarding the TAMU isolate is that it was obtained from a human who was infected and then it was cultivated in calves for the experiment. Thus, the TAMU isolate has cycled through calves less times than the other two isolates. This data may indicate the potential for parasite factors to contribute to infectivity more than host factors.

Table 2.1
Summary of *C. parvum* Oocysts Doses and Responses in Human Volunteers

Isolate	Collector	Derived Source	Number of Subjects	ID_{50} (# oocysts)	Mean Duration of Diarrhea (hours)
TAMU[1]	Dr. Snowden	Human[3]	14	9	94.5
UCP[2]	Dr. Ungar	Calf	17	1042	81.6
Iowa	Dr. Moon	Calf	29	87	64.2

Notes: [1]Texas A&M University isolate.
[2]Uniformed Services University of the Health Sciences, Bethesda, MD isolate.
[3]A veterinary student who was infected while performing necroscopy on an infected foal first passed the TAMU isolate. Dr. Charles Sterling at the University of Arizona then passaged the isolate in calves.
Data adapted from Okhuysen, et al., 1999.

Chappell, et al., (1999) investigated the infectivity rates of healthy humans with pre-existing anti-*C. parvum* serum immunoglobulin G (IgG). The Iowa isolate was used with an assumed ID_{50} of 132 oocysts. The ID_{50} for the healthy adults (17 subjects) was determined to increase 20 fold when compared to seronegative volunteers to 1,880 oocysts. When the subject group was further isolated to those subjects that shed oocysts, the ID_{50} increased 57 fold to 7,638 oocysts. This indicates that subsequent exposure will require larger numbers of oocysts to cause infectivity.

Also, fecal examination indicates that the number of oocysts shed may decrease with subsequent exposures. A previous study conducted by Okhuysen, et al., (1998) limited the question of re-infectivity to onset of symptoms after inoculation with 500 oocysts one year after primary exposure. A dramatic decrease in the reduction of volunteers shedding oocysts was observed from 63% (12 of 19) after the first exposure to 16% (3 of 19) after the second exposure. The rates of diarrhea were comparable between studies, but the production of anti-*C.parvum* IgG was markedly increased. Thus, an initial exposure is not sufficient to protect against clinical illness one year later, but the severity and intensity of infection decreased (Okhuysen, et al., 1998). This is positive to humans who suffer reinfection, but this will make it more difficult for medical and public health officials to detect and diagnose epidemiologically significant outbreaks.

2.3.4 Clinical Analysis

There are many different techniques being developed as new advancements in genetic decoding and microbiology evolve. Clinical diagnosis is commonly documented by:

- Serologic testing. This is the most common method of detection and focuses on antigen-antibody reactions by in-vitro testing. An example of this is the monitoring of anti *C. parvum* IgG concentrations in humans. IgG may be present for many decades and is not a good indicator of an acute infection.

- Microscopic observation of the oocyst in patient specimen (fecal matter).

- Detection of parasitic genetic material by PCR (polymerase chain reaction) has become available for detection. This method employs specific nucleic acid detection by a DNA probe and the polymerase reaction (uses an enzyme catalyst).

2.3.5. Epidemiological Case Studies

The first documented outbreak of waterborne cryptosporidiosis associated with drinking water occurred in July 1984 in Braun Station, Texas (a suburb of San Antonio). The largest outbreak occurred in April 1993 in Milwaukee, Wisconsin. However, epidemiological evidence has attributed outbreaks of cryptosporidiosis to a variety of sources (surface water and groundwater) undergoing a variety of treatment processes (conventional treatment, direct filtration, chlorination).

It is important to differentiate the water source for the outbreak because there have been documented incidents of waterborne cryptosporidiosis associated with recreational waters (i.e. community swimming pools). Consumption of treated drinking water is not the only source of waterborne cryptosporidiosis. Ingestion associated with contaminated recreational water continues to pose a large risk. Since 1971, the CDC, EPA, and the Council of State and Territorial Epidemiologists have maintained a collaborative surveillance system of waterborne-disease outbreaks (WBDOs). Epidemiological investigations of WBDOs attributed to *Cryptosporidium* introduce an interesting paradigm into how to truly protect public health. Appendix D provides a detailed summary of the epidemiological investigations of waterborne cryptosporidiosis in the United States with emphasis upon drinking water and recreational water sources.

2.3.5.1 Review of Documented Drinking Water Outbreaks

A WBDO is attributed to treated drinking water if the contamination by the etiological agent (*C. parvum*) occurs during treatment (from the source water collection through the distribution system) and not at the point of use. In a ten-year period (1984 – 1994), there were 10 documented outbreaks of waterborne cryptosporidiosis infecting an estimated 421,551 people in the United States from treated drinking water sources (see Table D.2.1). From 1995 to 1998, there have two documented outbreaks infecting an estimated 1,432 people associated with drinking water. Both of these resulted from fecal contamination entering the water source.

Details of the epidemiological investigation conducted after each outbreak highlights several key points. These points provide the basis of the "multi-barrier" approach to drinking water treatment that has become the doctrine of the industry and regulatory communities.

- Cryptosporidiosis occurred across all different water sources. Therefore, utilities and private systems are not immune from protecting their water source area.

- Single treatment mechanism was not sufficient to protect from disease, i.e. disinfection alone. Therefore, multiple systems in series should be employed.

- System optimization must be obtained. The large percentage of the cases may have been eliminated if processes that were built and operational were correctly managed.

- Filter backwash procedures may lead to an increase in concentration within the treatment plant. This was one of the treatment deficiencies stated in the Carrollton, GA, Talent, OR, and Milwaukee, WI epidemiological investigations. This has spurred the implementation of "filter-to-waste" procedures within the industry.

2.3.5.2 Review of Documented Recreational Water Outbreaks

Several cases of cryptosporidiosis have been documented in the United States that have been attributed to contaminated recreational water. These outbreaks usually are attributed to community swimming pools, public fountains, or contaminated lakes and streams. Evaluation of the epidemiologic investigation conducted between 1988 and 1998 for outbreaks associated with recreational water is presented in Table D.3.1. During this

timeframe, there have been 24 outbreaks infecting an estimated 11,902 people in the United States

2.4 Methods of Detection

Advances in microbiology and genotyping have aided the detection of oocysts in environmental (i.e. drinking water or natural water) samples. Preparation of environmental samples must follow the flow scheme presented in Figure 2e. The full range of methods will be addressed with specific attention given to flow cytometric cell sorting (FCCS) techniques and the United States Environmental Protection Agency (EPA) published method. All methods require a concentration step since the parasites are typically low in numbers and may be masked by other particulate matter in the sample.

Collection

↓

Separation (typically filtration)

↓

Concentration

↓

Purification

↓

Analysis (enumeration and viability)
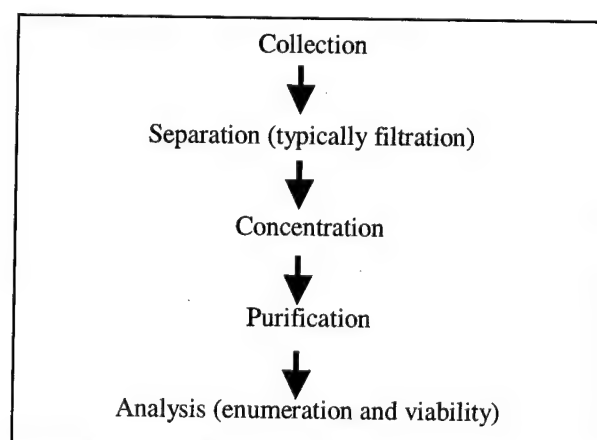
Figure 2e Environmental Sampling Flow Diagram

2.4.1 *The Spectrum of Methods*

"Mouse models have generally been suggested as the 'gold standard' for measuring oocysts infectivity (Rose, et al., 1999)." Although these models have an added value in a research setting, they are not very responsive in evaluating the potability and safety of a drinking water. Methodologies to determine the presence of oocysts in a sample may not accurately assess the viability (or infectivity) of the oocysts. Choosing a method that can provide an accurate, timely, affordable, and descriptive (infective or not) evaluation has been at the forefront of *Cryptosporidium* research.

It is also important to understand what each method is actually measuring. "Animal infectivity is a measure of the potential of the oocysts to complete their lifecyle in the host. In contrast, vital staining measures the presence or absence of metabolic activity. Excystation methods attempt to cause oocysts to respond to a biological stimulus (Black, et al., 1996)." This is an important consideration when attempting to correlate infectivity data and disinfection requirements. Black, et al. (1996) report an overestimated viability when using DAPI/PI vital dyes and excystation methods in comparison to animal infectivity (mouse) models.

Appendix B provides a listing of published methods investigated to measure *Cryptosporidium* and a comparison of the advantages and disadvantages associated with each method. This is provided to highlight the difficulty in choosing a method that is well suited for screening environmental samples. USEPA Method 1622 and FCCS are presented here in detail, as these are the basis of the regulatory approach and the sample analysis of this research, respectively.

There are numerous commercially available nucleic acid stains and anti-*Cryptosporidium* antibodies. Advantages and disadvantages of each evaluated product are presented in the literature with detailed method detection sensitivity. Two key developments analyzing nucleic acid stains and commercial antibodies are presented to highlight the importance of their applications.

Belosevic, et al. (1997) evaluated different nucleic acid stains. Using immunofluoresence assay (IFA) and nucleic acid staining will increase the specificity of the anti-parasite antibodies and the viability status. The advantage of using the nucleic acid dye is IFA staining is limited to the surface of the oocysts whereas the nucleic acid staining concentrates on the internal structures of the parasite. The ability of the nucleic acid to penetrate the oocyst cell wall is essential to decrease the number of "ghost" oocysts (no internal sporozoites) that are counted since they are not viable.

Hoffman, et al. (1999) evaluated four commercial antibodies. The antibodies that are chosen must be of high quality to ensure accurate and precise detection sensitivities. Some of the characteristics of antibodies that are critical are the avidity (strength of the antibody-antigen bonds), cross-reactivity with other species (particularly other *Cryptosporidium*

species as well as other parasites), and lot-to-lot variation. Another characteristic of importance is the fixation or fluorescence intensity. This is an important consideration to determine the percentage of live parasites.

Further analysis of purification procedures and the impact upon viability and infectivity have been considered. Slifko, et al. (2000) reported upon the impact of using various defatting agents (ethyl acetate and ethyl ether), density gradients (cesium chloride (CsCl), sucrose, and Sheather's solution) and separation techniques (IMS, bleach treatment (NaOCl), HCl). This group concluded, "purification procedures and oxidants do not appear to adversely affect oocysts infectivity (Slifko, et al., 2000)."

Brush, et al. (1998) investigated the impact of pretreatment and experimental conditions upon the electrophoretic mobility (measurement of net surface charge expressed as m^2/volt/sec) and hydrophobicity of oocysts. Negative surface charge at neutral pH was observed as reported in other studies. Hydrophobicity was reported to be a function of ionic strength (I). Two week old oocysts demonstrated strong adhesion at I = 0-10 mmol/L and moderate adhesion at I = 20 – 95 mmol/L. Hydrophobicity was also a function of oocyst age (high adhesion for 2 month old oocysts). These surface properties of the oocyst wall are crucial when evaluating detection methods and considering filter media and disinfectant chemicals.

2.4.2 USEPA Method 1622

The USEPA Method 1622 (Clancy, et al., 1999) evolved as a product of the Information Collection Rule (ICR). This evolution occurred as a method was needed to withstand a stringent interlaboratory, round robin testing program. Similar testing proved that previous methods were not very adequate due to variable recoveries, high false positive and false negative rates, and poor precision and accuracy. The ICR data was assessed using immunofluorescence assay (IFA).

Figure 2f depicts the sample process and evaluation as prescribed by Method 1622. One of the key factors involved within all methods is sample collection. This step is accomplished using a 1 μm nominal porosity wound polypropylene filter. The method detection limit (MDL) was conducted according to Appendix B, Part 136 of the Code of

Federal Regulations (CFR) and has been established at 4 oocysts per liter of water. The MDL determination procedure for microbiological analytes is currently under scrutiny by the USEPA and a future change may be implemented which would require a future validation of the method to be considered.

Collect 10 L of raw water in a clean carboy.

↓

Filter through filter capsule.

↓

Elute capsule with Laureth-12 buffer; centrifuge to a packed pellet.

↓

Perform immunomagnetic separation (IMS) using anti-*Cryptosporidium* IMS system.

↓

Stain on well slides with fluorescein isothiocyanate-4'-6'-diamidino-2-phenylindole (FITC-DAPI)

↓

Examine using epifluorescence and differential interference contrast microscopy

Figure 2f USEPA Method 1622 Flow Diagram
(Adapted from Clancy, et al., 1999)

2.4.3 Flow Cytometric Cell Sorting (FCCS)

The adaptation of flow cytometry in evaluating environmental samples has been well documented and widely practiced in both England and Australia. FCCS has been evaluated as a viable and effective alternative to IFA techniques in environmental samples as demonstrated by Hoffman, et al., (1999). The advantages of increased sensitivity, low cost per sample, decreased assay volume, and increased turnaround time warrant this technique as a plausible alternative.

Flow cytometry individually analyzes particles in a suspension. These individual particles or individual liquid droplets pass through the flow cytometer's laser beam producing several light scattering properties. The measure of forward scatter is directly correlated to the particle size (i.e., *C. parvum* is approximately 4-6 μm in diameter). The side-scatter or light deflected at a 90-degree angle correlates with the internal complexity of

the particle. The cell sorter has the ability to sort particles (an ultrasonic transducer that vibrates the sample stream separating it into droplets) of interest from other matter present in the liquid droplet. Thus, by implementing a "gating" criterion the particles can be separated within a defined sort region. This is accomplished when the instrument electrically charges the droplet carrying the particle and "pulls" the particle out of the stream by implementing oppositely charged deflection plates. Adjusting the wavelengths to correspond to the antibodies or nucleic acid stains conjugated to the sample, histograms can be generated corresponding to the amount of light emitted at the specified wavelength. This is a powerful tool that can determine not only enumeration, but also viability of the samples.

2.5 Regulatory Analysis

The United States Environmental Protection Agency (EPA) has responsibility for promulgation of regulatory statutes and laws under 40 CFR. Within this chapter, the EPA publishes regulations that are applicable to the drinking water industry. Microbial pathogen control and disinfection are addressed under a "family" of regulatory rules known as the Microbial /Disinfection Byproducts (M-DBP) Rules. These rules are the product of technical work groups addressing the trade-off of risks. The trade-off lies between the simultaneous control of microbial pathogens and the carcinogenic byproducts associated with the use of disinfectants. In order to address these risks and to provide "seamless" protection as these regulations are promulgated and implemented, the EPA has implemented the M-DBP Rules in several interim stages. Appendix C provides a detailed summary of these rules.

Currently, the Interim Enhanced Surface Water Treatment Rule (IESWTR) promulgated in 1998 requires 2-log removal. From the recent Agreement in Principle (AIP), Stage 2 of the Long Term ESWTR (LTESWTR2) may require more than 2-log removal and/or inactivation. From the AIP, it seems that future strategies will focus upon the technological refinement of filtration practices, implementation of new technologies (particular emphasis upon membrane filtration and ultraviolet (UV) light disinfection), and a "holistic" approach to incorporate multi-barrier treatment principles with regulatory compliance based upon source water quality.

2.6 Environmental Monitoring and Exposure Prevention

2.6.1 Monitoring

Environmental monitoring is very difficult to achieve. Current methods with regard to pathogen control and analysis are not compatible with "on-line" monitoring or rapid feedback. Development of refined laboratory procedures has evolved since the onset of the EPA imposed Information Collection Rule (ICR).

However, surrogate methods/measures and indicators are often employed to expedite the "OODA Loop" in making decisions. The OODA Loop is specified as the time it takes to start action; the shorter the cycle—the better. With pathogen detection, control and reaction, this loop must be shortened. However, based upon current methods of detection, associated costs, and knowledge of pathogens (which species are of concern), this loop can be very long (3 to 4 days or longer) and an outbreak may occur while this decision process is being satisfied.
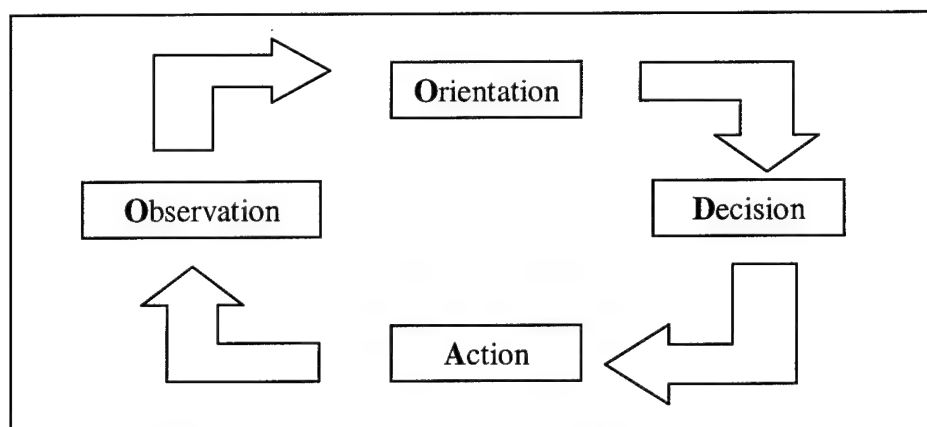
Figure 2g The "OODA Loop" Decision Making Cycle.
(Adapted from JA Starke Military Training, IOBC, 1992)

From this sort of approach to protecting populations from epidemic outbreaks, measures must be incorporated to reduce the time for the detection and analysis (**O**bservation and **O**rientation) and must provide public officials the maximum amount of time to make **D**ecisions and initiate **A**ction.

Monitoring within water sources, as for other waterborne pathogens, is very difficult. For this reason surrogate measurements that would indicate an increased chance of pathogen presence are monitored. Typically, turbidity and particle counts are employed as a measure

of the particles present in the water. Particle counts have the advantage to count particles that meet a certain size ("gate") criteria. Under the proposed Stage 2 M-DBP Agreement In Principle (AIP), there is an effort to try to correlate *C. parvum* monitoring with *E. coli* concentrations. This data is being generated by systems serving less than 10,000 people (biweekly sampling events). The ultimate goal would be to establish a tool to help assess appropriate times to increase plant surveillance and monitoring.

2.6.2 Preventive Measures

Preventive measures include both medical and life style considerations. These will only assist in decreasing the risk to sensitive population individuals.

- Medical:
    o Consult medical care at the earliest onset of gastrointestinal disease.
    o Drink plenty of fluids to maintain hydration and electrolyte levels.
- Life Style:
    o Increase personal hygiene practices, particularly after changing diapers, using the toilet, or before cooking/handling food.
    o Wash (or peel) all vegetables or fruits before eating them raw.
    o Minimize contact with water or food that may be contaminated.
    o Avoid drinking water from natural waters unless it has been filtered and chemically treated.
    o Boil water for 1 minute prior to ingestion.
    o Avoid swimming in pools (especially if infected or experiencing symptoms).

Section 3
Chlorine Disinfection

Disinfection is one of the treatment techniques within the water treatment field that contributes to establishing a "multi-barrier" approach to water treatment. The objective for disinfection is to limit the number of infectious organisms in drinking water. Disinfection does not 'remove the microorganism from the water; it only renders them ineffective to continue to reproduce or reduces their infectious capability. Chlorine disinfectant theory, current water treatment application, and disinfectant capabilities with respect to *Cryptosporidium* are presented in this section. Simple and readily applicable modeling to predict the effectiveness for log inactivation credit within a water treatment application is the desired goal of this research.

3.1 Chlorine Chemistry

Chlorine is the most commonly used disinfectant in the United States (approximately 80% according to an AWWA survey 1989). This is due to the strong oxidizing properties of chlorine. Chlorine can exist in six different valance states as listed in Table 3.1.

Table 3.1.
Valence States of Chlorine

Chlorine Form	Valence State
Chloride (Cl^-)	-1
Chlorine gas (Cl_2)	0
Hypochlorous acid (HOCl) Hypochlorite (OCl^-)	+1
Chlorite (ClO_2^-)	+3
Chlorine Dioxide (ClO_2)	+4
Chlorate (ClO_3^-)	+5

Chlorine used in disinfection comes in three different forms: chlorine gas (Cl_2), a liquid as sodium hypochlorite (NaOCl), and a solid pellet as calcium hypochlorite ($Ca(OCl)_2$). The reason other forms are not commonly used is due to cost and kinetic factors. Sodium hypochlorite was used for the experiments in this study.

Free Available Chlorine (FAC) is defined as the sum of the concentrations of molecular chlorine (Cl_2), hypochlorous acid (HOCl), and the hypochlorite ion (OCl^-).

The formation of FAC occurs with NaOCl by the following reactions:

$$\text{pH} \gg 7.5: \quad NaOCl + H_2O \rightarrow Na^+ + OCl^- + H_2O \quad\quad (3.1)$$

$$\text{pH} \ll 7.5: \quad NaOCl + H_2O \rightarrow Na^+ + HOCl + OH^- \quad\quad (3.2)$$
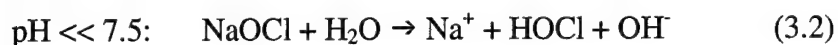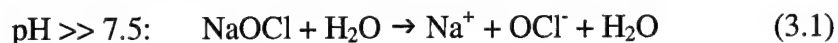
$$[FAC] = 2[Cl_2] + [HOCl] + [OCl^-] \quad\quad (3.3)$$

Note: All concentrations in Equation 3.3 are in moles/L

At pH 7.5 an equal amount of OCl^- and HOCl are formed. For every mole of NaOCl put into the system, one mole of FAC forms. For waters with a pH much lower than 7.5, a nearly instantaneous pH increase occurs. The extent of the increase depends on the initial pH and the alkalinity of the water. The concentration of FAC is typically reported in units of mg/L as Cl_2. The conversion from mg/L as NaOCl to mg/L as Cl_2 is given by the following:

$$\left(\frac{X\, mgNaOCl}{L}\right)\left(\frac{1mmoleNaOCl}{74.5mgNaOCl}\right)\left(\frac{1mmoleCl_2}{2mmoleNaOCl}\right)\left(\frac{70.9mgCl_2}{1mmoleCl_2}\right) = Y\frac{mg}{L}\, as\, Cl_2 \quad\quad (3.4)$$

To determine the distribution of free chlorine as a function of pH, the following equations are used.

$$K_a = \frac{[OCl^-][H^+]}{[HOCl]} = 10^{7.6} \quad\quad (3.5)$$

and

$$C_{Total} = [HOCl] + [OCl^-] \quad\quad (3.6)$$

where: K_a = equilibrium constant for the $HOCl/OCl^-$ system
C_{Total} = original amount of HOCl and OCl^- present

Combined available chlorine (CAC) is defined by the following chloramine species: monochloramine (NH_2Cl), dichloramine ($NHCl_2$), and trichloramine (NCl_3). On a molar basis, the CAC is defined as:

$$[CAC] = [NH_2Cl] + 2[NHCl_2] + 3[NCl_3] \quad\quad (3.7)$$

It follows that the total available chlorine, in moles/L, is the sum of the free available chlorine and the combined available chlorine.

$$[\text{Total Available Chlorine}] = [\text{FAC}] + [\text{CAC}] \tag{3.8}$$

Temperature and pH will impact the disinfecting strength of chlorine. It has been reported that hypochlorous acid is a stronger disinfectant than the hypochlorite ion (Finch, et al., 1997, Gyurek, et al., 1997). Thus, more favorable disinfectant conditions will exist below pH 7.5 at a constant temperature of 25°C. Temperature influences the pK_a value of the reaction, therefore impacting the distribution of chlorine amongst HOCl and OCl⁻. Typically, the pK_a will decrease as a function of increasing temperature (AWWA, 1999).

3.2 Chlorine Demand and Breakpoint Chemistry

Chlorine demand is defined as the difference between the applied chlorine dose and the chlorine residual measured after a specified time. Various constituents in the water and environmental conditions create this demand and consume the chlorine. Chlorine will react with other substances present in the water to form other chlorinated compounds. Some of these compounds are beneficial to disinfection (chloramination) whereas other byproducts such as trihalomethanes and haloacetic acids have an associated carcinogenic risk. Nonetheless, this demand must be accounted for when attempting to appropriately dose the water to achieve the desired disinfectant residual.

3.2.1 Ultraviolet Radiation

The direct presence of sunlight is an environmental condition that will create a chlorine demand by providing the required energy for the chlorine to react with the water. The light will decompose hypochlorous acid to the chloride ion (Snoeyink and Jenkins, 1980).

3.2.2 Oxidation of Reduced Substances

Chlorine has a significant oxidation potential and will oxidize reduced organic and inorganic substances when in contact with them. Some of the common substances that exert this demand in natural waters are natural organic matter (NOM), Mn(II), Fe(II), S(-II), and NO_2^-. The following two half-reactions represent this mechanism of chlorine decay for HOCl and OCl^-.

$$HOCl + H^+ + 2e^- \rightarrow Cl^- + H_2O \qquad (3.9)$$

$$OCl^- + H_2O + 2e^- \rightarrow Cl^- + OH^- \qquad (3.10)$$

3.2.3 Halogenation of Natural Organic Matter (NOM)

NOM is present in all surface waters and in many groundwater sources. The molecular composition of NOM cannot be characterized, but is expressed as the amount of organic carbon present in the water. NOM reacts with chlorine to form undesirable disinfection byproducts such as trihalomethanes (THMs). This is one of the primary concerns under the Disinfection Byproducts regulations.

3.2.4 Reactions with Ammonia

Chlorine will react with ammonia under a series of stepwise reactions to produce chloramines. The reactions governing the formation of chloramines are as follows:

Monochloramine:	$NH_{3(aq)} + HOCl \rightarrow NH_2Cl + H_2O$	(3.11)
Dichloramine:	$NH_2Cl + HOCl \rightarrow NHCl_2 + H_2O$	(3.12)
Trichloramine:	$NHCl_2 + HOCl \rightarrow NCl_3 + H_2O$	(3.13)

Chloramines have a beneficial effect as a weak disinfectant. When chlorine is added in the presence of ammonia, the resulting residual will be as CAC. Once the ammonia demand upon chlorine has been satisfied, the FAC will then form.

Chloramine Destruction: $\qquad 2NH_2Cl + HOCl \rightarrow N_2 + 3Cl^- + 3H^+ + H_2O$ (3.14)
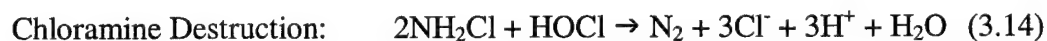
This complex set of reactions and kinetics is described as "breakpoint chemistry." This has the effect of adding a specified amount of chlorine to satisfy the ammonia demand (typically mass dose ratio of 7.5-10:1 $Cl_2:NH_4^+$-N is required) before FAC can be established. Breakpoint chemistry is outlined in more detail in AWWA, 1999.

3.2.5 Chlorine Demand Kinetics

The constituents of chlorine demand are largely unknown, as are their rates of reaction with chlorine. Many models have been developed to try to mathematically and empirically represent this relationship. The least complex of these models is simple first-order decay model, represented in Equation 3.15.

$$\frac{dC}{dt} = -k_d C \qquad (3.15)$$

where: C = residual concentration.
k_d = first-order rate constant (min^{-1}).

It may be necessary to depict the chlorine demand through more complex decay models. A simple second-order decay model is shown in Equation 3.16.

$$\frac{dC}{dt} = -k_d C^2 \qquad (3.16)$$

This demand may be best represented by a combination of 1st and 2nd order decay models. A model relating the demand to 2nd order overall, but 1st order with respect to two different demanding substances may also be valid as shown in Equation 3.17.

$$\frac{dC}{dt} = -k_d C_A C_B^2 \qquad (3.17)$$

In order to depict this demand and accurately express the rate constant, it will be paramount to describe this demand as a function of time. Nonetheless, the chlorine demand

expressed as a rate constant will be unique to each specific water type and may vary with water characteristics. Appendix F provides derivations of these models for a CMFTR. These models can be used to predict chlorine concentration as a function of detention time.

3.2.6 Disinfection Kinetics Disinfection Kinetics

Disinfection kinetics are not completely understood. There are several models, each attempting to describe characteristics of the reactor system and the disinfectant used.

3.2.6.1 Chick's Law

In 1908, Harriet Chick described disinfection as a process similar to that of a chemical reaction. Chick's Law is a first-order rate law that defines the disinfectant and the microorganisms as the reactants. The reaction velocity (i.e., the number of microorganisms destroyed per time interval) is proportional to the number of microorganisms remaining.

$$\frac{dN}{dt} = -k_i N \tag{3.18}$$

where: k_i = inactivation rate constant.
N = concentration of viable organisms.

3.2.6.2 Chick-Watson Law

Watson modified the model to account for the disinfectant concentration (Watson, 1908). The resulting rate equation is known as the Chick-Watson Law:

$$\frac{dN}{dt} = -k_i C^n N \tag{3.19}$$

where: C = concentration of the disinfectant.
n = order of the reaction with respect to the disinfectant.

Integration of this law predicts an exponential decay for batch reactors.

$$\ln\left(\frac{N}{N_0}\right) = -kC^n t \tag{3.20}$$

where: N = concentration of viable microorganisms at time = t.
N_0 = concentration of viable microorganisms at time = 0.

3.2.6.3 Deviations

There are two common deviations from the Chick-Watson Law known as the shouldering effect and the tailing-off effect. The shouldering effect occurs due to a time lag of inactivation at the onset of disinfection. This effect is typified with organisms that form colonies and more than one cell must be inactivated to achieve inactivation of a colony. The tailing-off effect occurs when the rate of inactivation decreases as a function of time. There are several possible reasons for this effect including: conversion to a resistant form, clumping of subpopulations, variations in the dose received, and variable sensitivity to the disinfectant (AWWA, 1999). Models that consider these deviations have been better able to predict the inactivation of organisms for batch systems (Gyurek, et al., 1997; Finch, et al., 1993; Hom, 1972; Severin, et al., 1984). Figure 3a represents these deviations.

Line A: Chick-Watson
Line B: Shouldering Effect
Line C: Tailing Effect

$\log(N/N_0)$

Detention Time
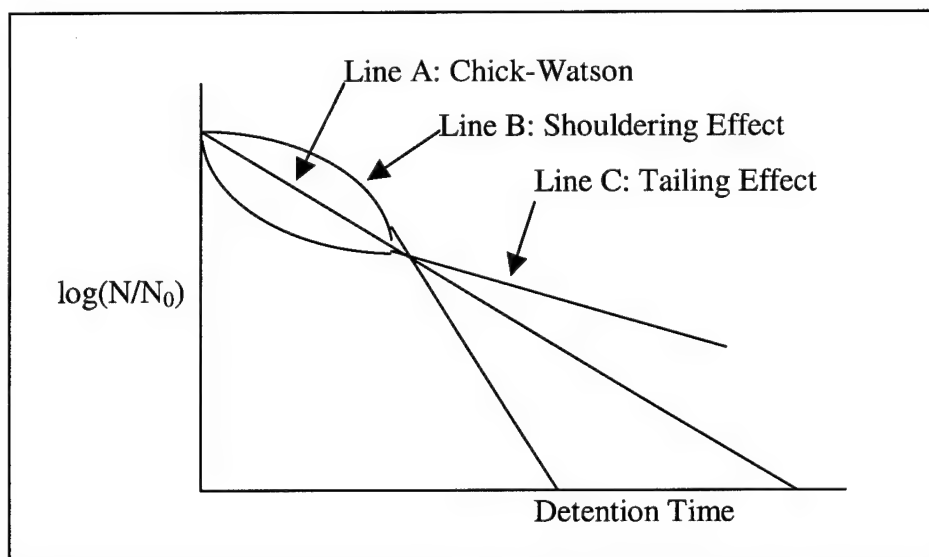
Figure 3a Chick-Watson Model and Its Deviations (AWWA, 1999)

3.3 Reactor Hydraulics

3.3.1 Reactor Types

There are three types of ideal reactors: batch reactors, plug flow reactors (PFR), and completely mixed flow-through reactors (CMFTR). Characteristics and inherent assumptions of each are listed below.

Batch Reactors:

- Water enters system in batches, i.e., fill and draw cycles.
- No material crosses system boundaries.
- Composition changes with time. .
- Common in lab settings, seldom found in practice at large scales.

Plug Flow Reactors:

- High length-to-width ratio.
- No mixing in the axial direction, but complete mixing in radial direction.
- Constant flow of water into and out of system, only at steady-state.
- At steady-state, composition does not change with time.

Completely Mixed Flow-Through Reactors (CMFTR):

- Uniform composition.
- Constant flow of water into and out of system, only at steady-state.
- At steady-state, composition does not change with time.

Generally, a larger volume of water is required for a CMFTR to produce the same concentration as a PFR. In a laboratory setting, we can come very close to constructing a CMFTR, but we have great difficulty in constructing a PFR. CMFTRs may be more resilient to sudden changes in influent conditions.

3.3.2 Ideal Rectors

Mass balance calculations are the basis for all reactor designs. The general mass balance equation is given in Equation 3.21.

$$\left(\frac{mass}{time}\,accumulated\right) = \left(\frac{m}{t}\,in\right) - \left(\frac{m}{t}\,out\right) + \left(\frac{m}{t}\,produced\right) - \left(\frac{m}{t}\,consumed\right) \qquad (3.21)$$

The mass balance relation can be used to determine the hydraulic residence of the reactor. The hydraulic residence time is defined as the time each element of the fluid spends

within the reactor. The nominal hydraulic residence time (HRT), $\tau_{nominal}$, is defined in Equation 3.22.

$$\tau_{nominal} = \frac{V}{Q} \tag{3.22}$$

where: V = volume of the reactor.

Q = volumetric flow rate (volume per time).

Mass balance analysis may be performed on a CMFTR depicted in Figure 3b with respect to either the disinfectant or the microorganisms. Equations 3.23 and 3.24 describe the general mass balance with respect to disinfectant decay and microoogrganism inactivation, respectively.
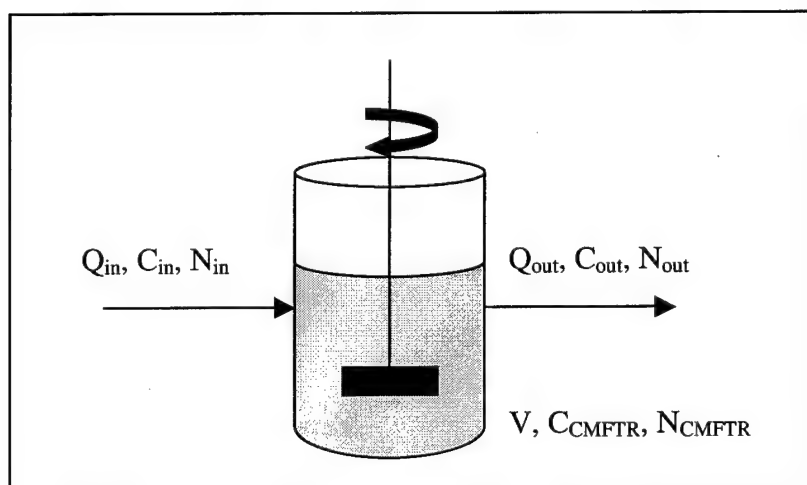
Figure 3b CMFTR Schematic.

$$QC_{in} - QC_{out} - Vr_d = V\left(\frac{dC_{out}}{dt}\right) \tag{3.23}$$

where: C_{in} = concentration of disinfectant entering the reactor (mg/L).

C_{out} = concentration of disinfectant leaving the reactor (mg/L).

r_d = rate of disinfectant decay within the reactor (mg/min-L).

$$QN_{in} - QN_{out} - Vr_i = V\left(\frac{dN_{out}}{dt}\right) \tag{3.24}$$

where: N_{in} = concentration of microorganisms entering the reactor (oocysts/L).
N_{out} = concentration of microorganisms leaving the reactor (oocysts/L).
r_i = rate of microorganism inactivation within the reactor (oocysts/min-L).

Typically, analysis is performed assuming steady-state conditions have been achieved. Such conditions are achieved when the concentration of substances within the reactor are not changing with time. The CMFTR can be designed using the following steady-state equations.

$$\tau_{no\,min\,al} = \frac{V}{Q} = \frac{C_{in} - C_{out}}{-r_d} \tag{3.25}$$

where: $-r_d = -k_d C_{out}$ or $-k_d C_{out}^2$ or other appropriate model.

$$\tau_{no\,min\,al} = \frac{V}{Q} = \frac{N_{in} - N_{out}}{-r_i} \tag{3.26}$$

where: $-r_i = -k_i C_{out}^{\,n} N_{out}$ or other appropriate model.

3.3.3 Nonideal Reactors

Deviations from ideal conditions will be experienced due to the creation of stagnant zones (dead space), channeling of fluid (short-circuiting), and fluid recycling within the reactor. Ultimately, these deviations result in a continuous distribution of residence times within the reactor. Models, based upon tracer study data, can be used to estimate reactor performance. These models attempt to use empirical data to account for deviations from the assumption of no axial dispersion in the PFR reactor and deviations from homogenous concentration within a CMFTR.

The plug flow with dispersion model attempts to account for axial dispersion. It mathematically represents this anomaly with the dispersion coefficient (D). The general equation for this model is given in equation 3.27 and is applicable to any reactor system. Within this model, D_{PFR} will approach 0 whereas D_{CMFTR} will approach infinity.

$$\frac{dC}{dt} = -\left(\frac{Q}{A}\right)\left(\frac{dC}{dt}\right) + \left(\frac{d^2C}{dl^2}\right) + r_c \qquad (3.27)$$

where: C = concentration of disinfectant.
t = time.
Q = flow rate.
A = cross-sectional area.
l = axial distance.
D = dispersion coefficient.
r_c = net rate of production of disinfectant.

The CMFTRs in series model incorporates a number (Z) of equal sized tanks in series. This model is applicable to any reactor system with Z approaching infinity for a PFR and Z approaching 1 for a CMFTR. This approach is very useful in using CMFTR lab data to predict PFR conditions. The CMFTR in series model can be quite complex dependent upon the order of the disinfectant decay or inactivation rate. Equations 3.28 and 3.29 depict the CMFTRs in series model for first-order disinfectant decay and Chick-Watson inactivation kinetics under steady-state conditions, respectively. Currey (2001) presents a full derivation of these equations.

$$\frac{C}{C_0} = \left(\frac{1}{k_d\tau/Z + 1}\right)^Z \qquad (3.28)$$

$$\frac{N_z}{N_0} = \sum_{i=1}^{z}\left(\frac{1}{1 + k_i C_i^n \tau}\right) \qquad (3.29)$$

Assuming that the disinfectant concentration is the same through each reactor, Equation 3.30 can be simplified. This would only occur under demand-free conditions.

$$\frac{N}{N_0} = \left(\frac{1}{1 + k_i C^n \tau/Z}\right)^Z \qquad (3.30)$$

3.3.4 Tracer Studies.

Tracer studies can be conducted to characterize the continuous distribution of residence times within a given reactor configuration. An ideal tracer will be chemically inert, behave as a dissolved substance, and be easy to measure. The tracer is injected at the inlet and the tracer concentration is measured at the outlet of the reactor. There are two common tracer studies implemented: pulse-dose (the tracer is injected instantaneously into the influent stream) and step-dose (the tracer is injected at a constant concentration into the influent stream).

This data is then analyzed using statistical relations by transforming the data. Levenspiel (1962) provides a detailed presentation of the transformation plots of the data. From this analysis, a distribution of residence times can be obtained. These are usually defined in terms of percentile residence times (i.e., t_{10}, t_{90}, t_{95}, etc.) and the mean residence time (mean t). The percentile residence time is defined as the time at which a given percentage of the fluid has passed through the reactor. For example, at t_{10}, 10% of the fluid has passed through the reactor and 90% of the fluid remains in the reactor. This t_{10} can also be stated as the time at which 10% of the fluid has a smaller residence time and 90% of the fluid has a larger residence time. The t_{10} value is used in regulatory compliance.

3.4 Disinfection Log Inactivation Models

Table 3.2 summarizes the rate equation and CMFTR model for each. The derivation and experimental basis for each of the five accepted models is well documented in the literature. The models for the CMFTR are derived from a mass balance on viable organisms. The mass balance on a CMFTR (Figure 3b) assumes Chick-Watson kinetics, no growth within the reactor, and achievement of steady-state conditions.

Mass Balance: $0 = Q_{in} \; N_{in} - Q_{out} \; N_{out} - (k \; C_{out}^{n} \; N_{out})V$ (3.31)

Based upon this relationship, Figure 3c shows the Chick-Watson Model and deviations for a CMFTR.

Table 3.2
Disinfection Log Inactivation Models

Model	Rate Equation	CMFTR Model	Reference
Chick	$\dfrac{dN}{dt} = -kN$	$\ln\left(\dfrac{N}{N_0}\right) = -\ln(1 + k\tau)$	Chick, 1908
Chick-Watson	$\dfrac{dN}{dt} = -kNC^n$	$\ln\left(\dfrac{N}{N_0}\right) = -\ln(1 + kC^n\tau)$	Watson, 1908
Rational	$\dfrac{dN}{dt} = -kN^xC^n$	$\ln\left(\dfrac{N}{N_0}\right) = -\ln(1 + kN^{x-1}C^n\tau)$	Haas, 1995
Hom	$\dfrac{dN}{dt} = -kNC^nt^m$	$\ln\left(\dfrac{N}{N_0}\right) = -\ln(1 + kC^n\tau^{m+1})$	Hom, 1972
Modified Hom	$\dfrac{dN}{dt} = -kmNC^nt^{m-1}$	$\ln\left(\dfrac{N}{N_0}\right) = -\ln(1 + kmC^n\tau^m)$	Finch, 1993 Gyurek, 1997

Notes: Adapted From: Harrington, 1999.
N_0 = initial concentration of viable microorganisms entering a CMFTR.
N = concentration of viable microorganisms leaving a CMFTR.
C = concentration of the disinfectant leaving a CMFTR.
t = reaction time in a batch reactor.
τ = mean hydraulic residence time in a CMFTR.
k = inactivation rate constant
n = order of inactivation reaction with respect to the disinfectant (coefficient of dilution).
x = order of inactivation with respect to the microorganism.
m = an empirical fitting factor.
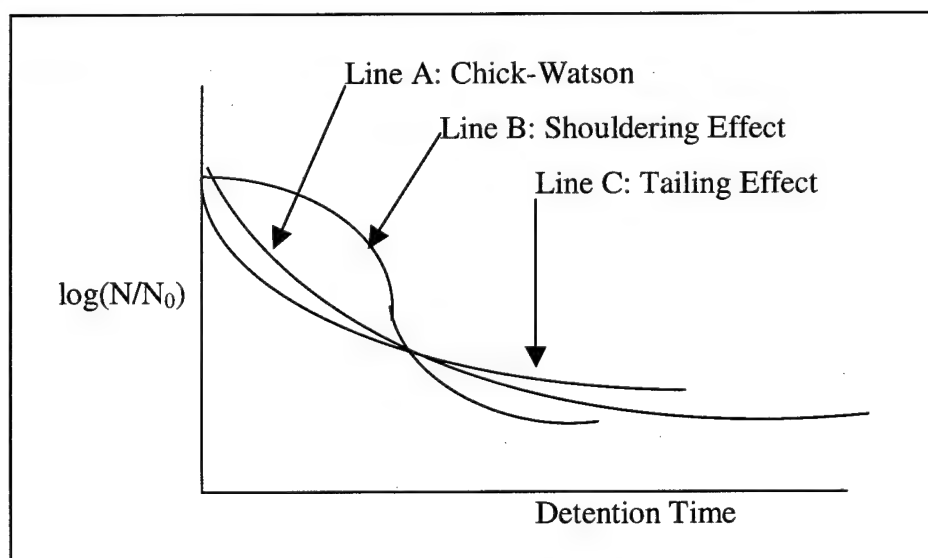
Figure 3c Chick-Watson Model and Its Deviations For a CMFTR

3.5 CT Concept

3.5.1 Definition

The number and viability of infectious microorganisms cannot be monitored to provide this data in a timely manner to make operational decisions. However, the Chick-Watson model shown in Table 3.4.1 shows that the product of C and τ can serve as a surrogate means of estimating process performance (assuming $n = 1$ and k_d is known). This is advantageous because water utilities can effectively, timely, and practically monitor disinfectant residuals. Under the Surface Water Treatment Rule (SWTR), utilities are required to meet disinfection requirements using the CT concept. CT is defined in Equation 3. 32.

$$CT = C \times t \tag{3.32}$$

where C = residual concentration of disinfectant (mg/L)
t = contact time (min)
CT (mg-min/L).

This concept ensures that inactivation will increase as the CT value increases. Changing the disinfectant concentration or the contact time can control CT values.

3.5.2 Regulatory Considerations.

The U.S. Environmental Protection Agency (EPA) has defined CT values for regulatory compliance. The CT value is defined as the residual concentration of disinfectant (C_{OUT}) leaving a reactor multiplied by the 10th percentile contact time (t_{10}) of the reactor. A conceptual view of t_{10} is that 10% of the water moving through the reactor spends less time in the reactor than given by the t_{10} value. This means that 90% of the water is in contact with the disinfectant for a time greater then or equal to the t_{10} value.

The existing regulations have specified CT values for up to 3-log inactivation (99.9%) of *Giardia lamblia* and up to 4-log (99.99%) inactivation of enteric viruses. Within the regulations, removal credit is received for implementing other treatment processes. For conventional treatment (coagulation, flocculation, sedimentation, and filtration), a utility may receive 2.0 to 2.5-log removal credit for *Giardia lamblia* and 1.5 to 2.0-log removal credit for enteric viruses. These credits are then applied to the overall requirement for 3-log

removal and/or inactivation of *Giardia lamblia* and 4-log removal and/or inactivation credit for enteric viruses. These credits reduce the CT required by disinfection. There are published tables that are used to assist in determining the required CT value. These values are based upon demand-free waters with a first-order Chick-Watson estimation of the microbial decay rate. Therefore, the published CT values are independent of initial microorganism concentration and the concentration of chlorine demanding substances. As described in Appendix C, there are no published regulatory CT values for *Cryptosporidium*. It is important to note that the proposed Agreement In Principle (2000) may require 3.0 to 5.5-log inactivation and/or removal for *Cryptosporidium*. Given expected credit for conventional treatment, utilities may use disinfectants to achieve up to 2.5-log inactivation. Appendix C provides a more detailed analysis of the regulatory requirements.

3.5.3 Cryptosporidium Case Studies.

There have been numerous published chlorine inactivation experiments conducted within the past ten years. These studies confirm that chlorine inactivation of *Cryptosporidium* is ineffective at CT values typical of municipal water treatment systems (Campbell, et al., 1992; Lorenzo-Lorenzo, et al., 1993; Korich, et al., 1990; Fayer, 1995; Finch, et al, 1997; Gyurek, et al., 1997; Venczel, 1997). In order to achieve 2-log inactivation, a CT value of 3700 mg-min/L was required at pH 6 (Driedger, et al., 2000).

However, the majority of the reported experiments involve the use of buffered, demand-free waters in small batch reactors. The use of Flow Cytometry Cell Sorting has not been widely used in conjunction with these experiments as this research has used a variety of methods (mouse infectivity and vital dye staining). Therefore, potential shortcomings in these studies may be the lack of characterization of the effects from natural waters, chlorine-demanding substances, large volumes of water, and flow-through systems. Figure 3d represents the published *Cryptosporidium* log inactivation data as a function of CT. The published data may not be compared independent of the effects of different measurement techniques. Gyurek (1997) and Finch (1997) used mouse infectivity, whereas Driedgar (2000) used *in vitro* excystation.
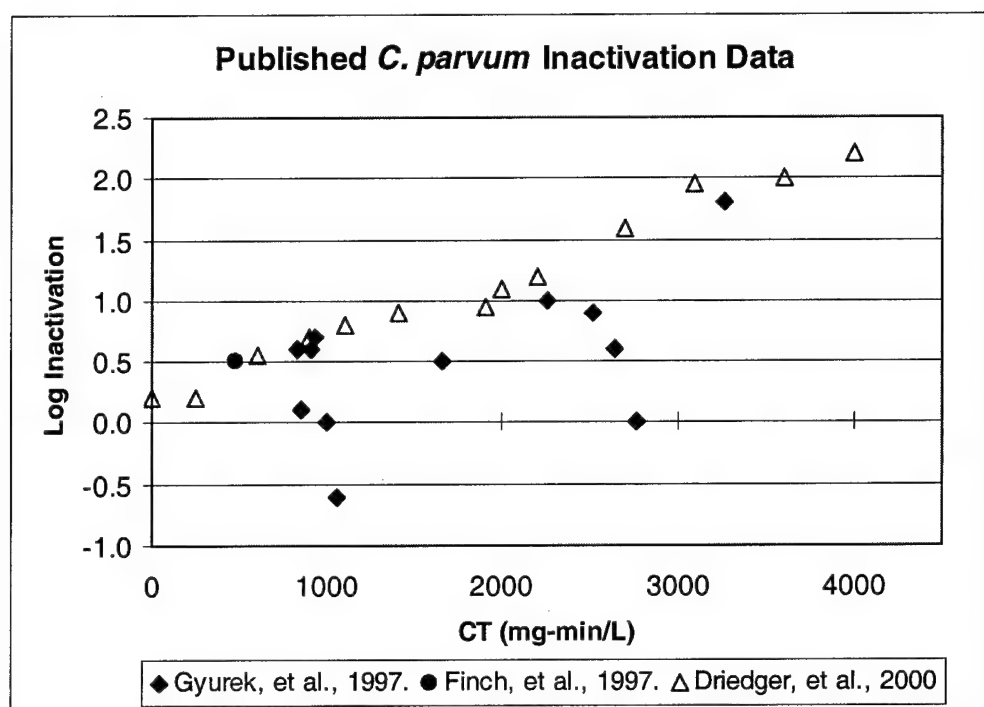
Figure 3d Published *C. parvum* log inactivation data versus CT (pH 6 and 20 to 22°C)

Section 4
Experimental Procedures

The evaluation of the influence of chlorination upon the inactivation of *C. parvum* was conducted under a variety of water quality parameters and various CT values. Enumeration and viability of the *C. parvum* oocysts was accomplished using flow cytometric cell-sorting (FCCS) techniques.

4.1 System Description

A series of bench-scale, steady-state, completely-mixed flow-through reactors (CMFTR) were used to obtain a variety of representative CT values. Figure 4a depicts the overall experimental flow scheme. A detailed analysis of the behavior of the system was conducted using a sodium chloride tracer study. The data for this study are presented in Appendix G with a summary of the results presented in Section 5.

Note:
9 Reactors Total:
#1-3: 2L
#4-6: 1L
#8-9: 0.5 L

⊗ HOCl and *C. parvum* Sampling Point

⟶ *C. parvum* Feed System

---▶ HOCl Feed System
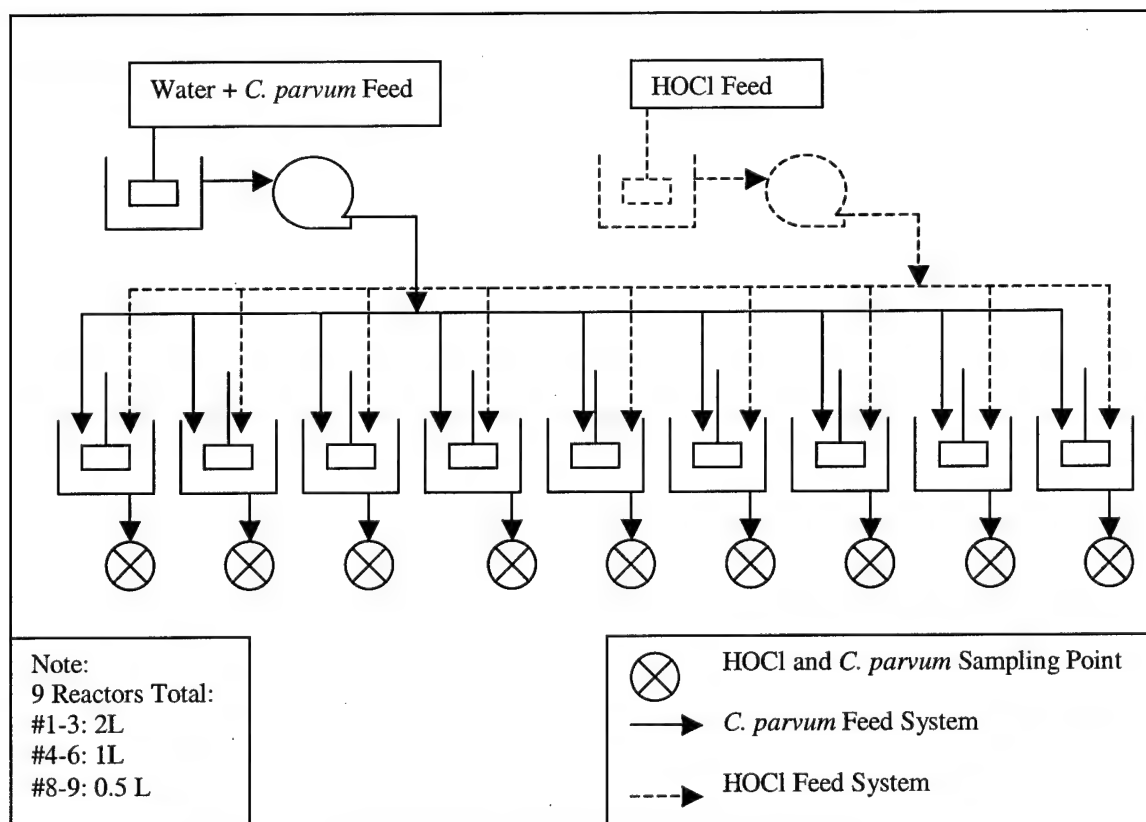
Figure 4a. Experimental Flow Scheme

4.2 Reactor Design

Glass filtering flasks equipped with a sidearm (Fisher Scientific, Pittsburgh, PA) were operated as the CMFTRs. Different nominal detention times were achieved by varying the volume of the reactor; 580 mL, 1230 mL, and 2250 mL volumes were used. The individual reactor design is depicted in Figure 4b. Each reactor was equipped with a stainless steel needle (Hamilton Hypothermic Needle, Hamilton Company, Reno, NV) and glass syringe (Micro-mate 10cc Interchangeable Syringe, Popper & Sons, Inc., New Hyde Park, NY) to serve as the collection port. The reactors were sealed at the top with solid silicone stoppers (Fisher Scientific, Pittsburgh, PA). Glass tubing was used for influent and effluent flow. The influent tube allowed for water to be introduced at the bottom of the reactor while the effluent was collected from the top of the reactor. Influent and effluent tubing was PharMed brand tubing (Cole Parmer, Vernon Hills, IL) and the chlorine tubing was C-Flex brand tubing (Cole Parmer, Vernon Hills, IL).

1. Combined *C. parvum*/HOCl Feed
2. Effluent
3. Teflon-coated Stopper
4. Zero Headspace
5. Erlenmeyer Flask with Sidearm
6. Teflon-coated Magnetic Stir Bar
7. Magnetic Stir Plate
8. Syringe with Sampling Needle
9. Luer-lock Valve
10. Septum

Note:
Glass Tubing
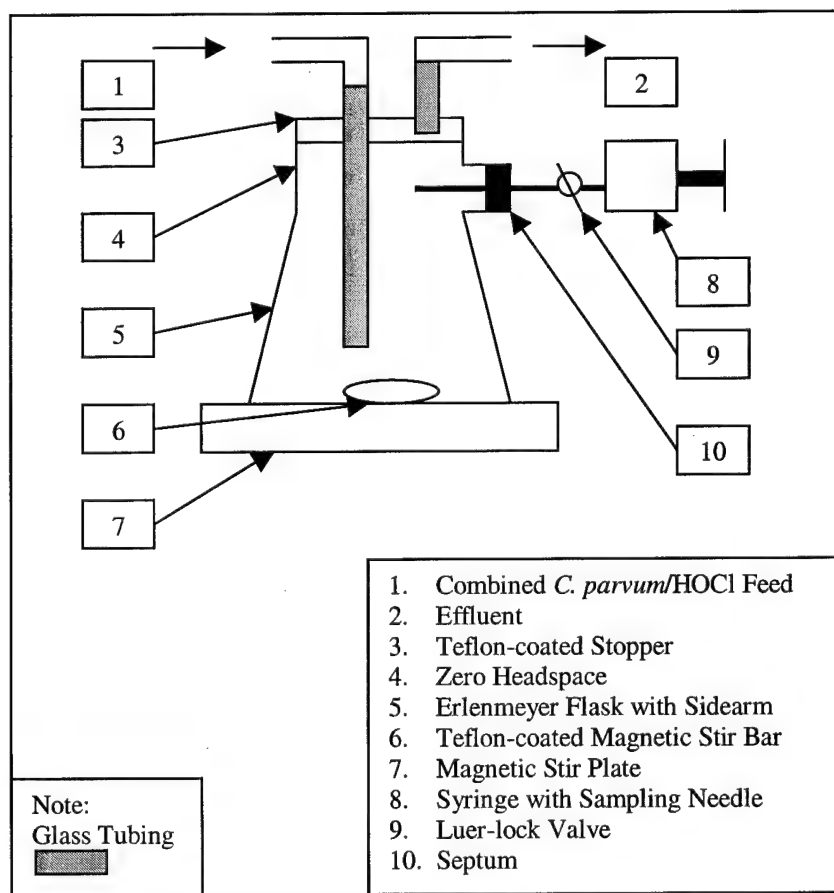
Figure 4b. Schematic of the CMFTR Design

Teflon-coated magnetic stir bars were used to achieve completely mixed conditions within the reactors. All stoppers incorporated a designed "notch" to eliminate headspace and therefore decrease disinfectant volatility. Covering the reactors with aluminum foil minimized photodecomposition of the disinfectant.

4.3 Water Source

Water used for all experiments was derived from two sources. The "raw" water refers to water that was pumped directly from Lake Mendota into the UW-CEE Water Treatment Pilot Plant. This water received no physical/chemical treatment with the exception of passing through the screen (1/8-in mesh) housed on the influent pump. The "treated" water was the filtered effluent from one treatment train within the pilot plant (Burt Process Equipment, Inc., Hamden, CT). A schematic of the treatment train is depicted in Figure 4c.
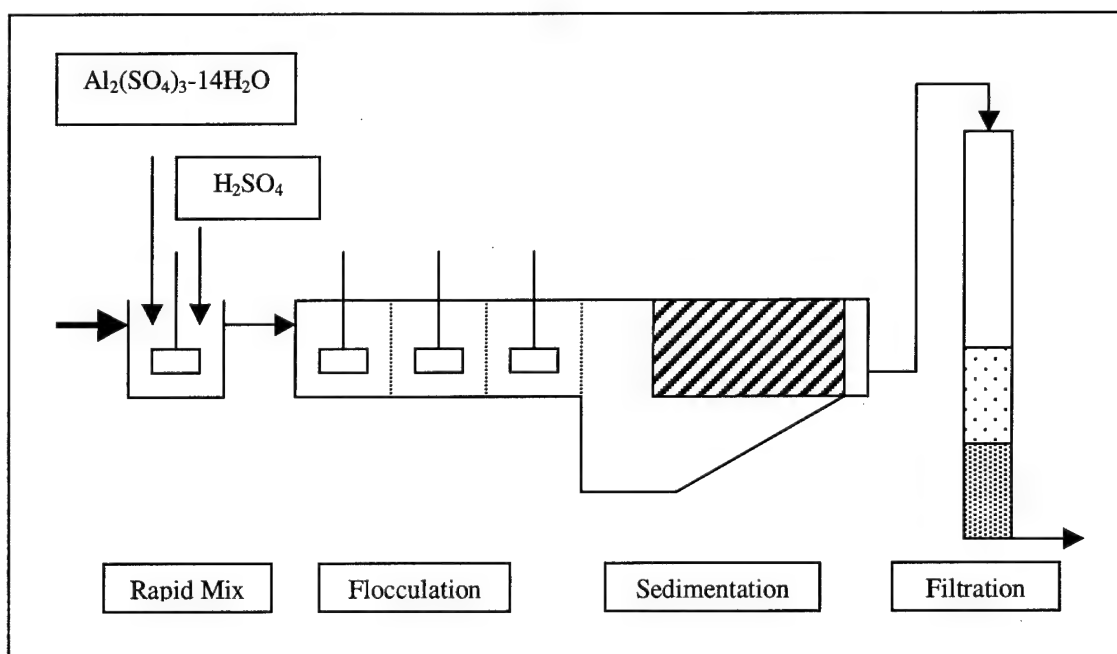
Figure 4c UW CEE Water Treatment Pilot Plant Schematic

4.4 Pilot Plant Operating Conditions

The raw water was subjected to the following physical/chemical treatment processes: rapid mix; 3-stage flocculation; sedimentation (via settling tubes); and filtration (dual media

filters). Raw Lake Mendota water was pumped to a constant head tank before distribution to the treatment train at a flow rate of 0.4 gallons per minute (1.65 L/min). Aluminum sulfate (Fisher Scientific, Pittsburgh, PA) was fed from a 55 gallon (200 L) feed tank (Nalgene Lightweight Graduated Cylindrical HDPE Tank, Fisher Scientific, Pittsburgh, PA) and sulfuric acid (Fisher Scientific, Pittsburgh, PA) was added with a target pH adjustment of 6.0 in the rapid mix tank. Rapid mixing was maintained at a speed of 800 rpm. The water then flowed through three flocculation chambers with tapered mixing speeds of 55, 31, and 22 rpm, respectively. The water then flowed into the sedimentation tank, up through inclined tube settlers, and over a weir. The water was then pumped to a filtration constant head tank. Water was then filtered over a dual media (anthracite/sand) filter at a rate of 11 gallons per hour (3.74 gpm/ft^2).

Operation of the plant included daily flow, head loss, pH, and turbidity monitoring to ensure steady-state conditions were maintained. Samples taken three times per week were analyzed for TOC, DOC, and UV_{254}. Typical water quality characteristics, reported as average values, of the water used during this study are listed in Table 4.1.

Table 4.1
Lake Mendota Water Quality Characteristics

Parameter	Raw Water	Filtered Water
pH	8.1	6.3
TOC	4.9	3.1
DOC	5.0	3.2
UV_{254}	0.077	0.039

The train was drained and cleaned once per week to maintain particle-settling conditions, jar tests were conducted on a regular basis to optimize coagulant doses, and filters were backwashed when the head loss was greater than 50 inches or filter effluent turbidity was greater than 0.5 NTU. Typical alum doses applied during the course of this study are listed in Table 4.2.

Table 4.2
Typical Pilot Plant Alum Doses

Date	Alum Dose (mg/L)
April 1 – July 2, 2000	30
July 3 –19, 2000	50
July 20 – February 4, 2001	70
February 5, 2001 – May 2001	50

4.5 Experimental Procedure

The same protocol was used for each experimental run. This involved a coordinated effort with the Wisconsin State Lab of Hygiene in order to ensure all Biohazard safety and quality control issues were achieved prior to every experiment. Table 4.3 provides a summary of all required protocol steps and Appendix F provides a detailed experimental checklist and timeline.

Water spiked with *Cryptosporidium* was fed to the reactors from a 55 gallon (200 L) feed tank (Nalgene Lightweight Graduated Cylindrical HDPE Tank, Fisher Scientific, Pittsburgh, PA) that was continuously stirred to provide completely mixed conditions within the tank and to minimize settling of *Cryptosporidium* in the tank. The feed water flow rates were set to achieve nominal detention times of approximately 30, 60, and 90 minutes. The feed water consisted of either raw or treated Lake Mendota water collected the day prior to the experiment with typical characteristics previously described.

Chlorine stock solutions were fed to the reactors at flow rates ranging from 1-2% of the total system flow. Chlorine stock solutions were prepared one day prior to the experiment using sodium hypochlorite (6% available chlorine, Fisher Scientific, Pittsburgh, PA) and Milli-Q water (18.3 μmho).

Once the water and chlorine flows into the reactors were confirmed, the system was run until steady-state was achieved. The time to achieve steady-state was determined by tracer study data. Once this time was achieved, disinfectant residual samples were collected to validate that steady-state had actually been attained. These samples were analyzed for free chlorine, monochloramine, and dichloramine residual concentrations within ±0.3 mg/L.

Samples were collected throughout the experiment to obtain a 50 mL sample volume. Initially, samples were taken from the feed tank to measure background *Cryptosporidium* concentrations prior to the "spiking" of the water. After the spiking, samples were collected from the feed tank at the start, midpoint, and end of each experiment. These samples ensured that uniform mixing conditions were maintained and oocyst settling did not occur. Once steady-state was attained, composite samples were collected from each reactor. The composite sample represented a 40-minute period of steady-state operation and was comprised of five 10 mL samples collected at 10 minute intervals. Each 10 mL sample was preloaded with sodium thiosulfate (Fisher Scientific, Pittsburgh, PA) to quench any chlorine residual and prevent further disinfection. Samples were collected in 50 mL polypropylene conical tubes and stored at 4°C until analysis.

Table 4.3
Experimental Protocol

Event	Remarks	Responsibility
Oocyst Preparation[1]	• Receive oocysts from M. Marshall at University of Arizona[3]. • Count oocyst for initial concentration. • Microscopically screen oocyst for potential contamination.	State Lab
Apparatus Set-Up[1]	• Ensure set-up completed IAW Section 4.1. • Establish and measure flow rates.	CEE Researchers
Chlorine Demand Experiment[3]	• Verify the actual chlorine demand of the experiment water by running the system without oocysts for the duration of the experiment. • Establish chlorine demand curve.	CEE Researchers
Water Quality Parameters and Stock Solutions[1]	• Measure water quality as per Section 4.4. • Prepare stock solutions for disinfectant IAW chlorine demand curve.	CEE Researchers
Biosafety[1]	• Ensure all required items are inventoried, on-hand, and operational. • Notify all key personnel of experimental timeline. • Cordon laboratory as Biohazard Level 2 Area.	CEE Researchers

Experiment[2]	• The sequence of events followed:
	  o Verify flow rates (water and chlorine)
	  o Measure chlorine stock solutions
	  o Add oocysts to feed tank
	  o Add oocysts to control beaker[5]
	  o Measure chlorine residual and flow rates IAW timeline provided in Appendix C
	  o Verify steady state conditions
	  o Sample IAW timeline provided in Appendix C.
	  o Measure final chlorine flow rates.
		CEE Researchers

Biosafety[2]	• All laboratory workspace was treated as a Biohazard Level 2 Hazard and was cleaned IAW disinfection protocol provided in Appendix C. • All laboratory equipment was treated as a Biohazard Level 2 Hazard and was autoclaved and washed.	CEE Researchers
Sample Evaluation[4]	• Samples were transported and stored at the State Lab of Hygiene. • Samples were then evaluated as per Section 4.5.	State Lab

Notes: [1]Conducted the day prior to the experiment
[2]Conducted the day of the experiment
[3]Conducted prior to the experiment
[4]Conducted after the experiment (typically within 48 hours)
[5]A control beaker placed upon a magnetic stir plate was measured concurrently with the experiment to test for any deviations occurring in the feed tank or reactors
IAW = in accordance with

4.6 Water Quality Analysis Methods

Water quality parameters were measured to describe the water prior to and during each experimental run. Table 4.4 describes the water quality parameters tested and the method used. Water quality data are described in Section 5.

Table 4.4
Summary of Analytical Methods

Parameter	Method Title	Standard Method Number[1]	Remarks
Turbidity	Nephelometric	2130 B	Turbidimeter
Alkalinity	Titration	2320 B	HCl used
Hardness	EDTA Titrimetric Method	2340 C	HACH Test Kit
Conductivity	Electrometric	2510	Conductivity Meter
Temperature	Mercury Thermometer	2550 B	
Chlorine	DPD Ferrous Titrimetric	4500-Cl F	
pH-value	Electrometric	4500-H$^+$ B	Denver Instruments Basic
Ammonia		4500-NH$_3$	HACH Test Kit
Total Organic Carbon	Combustion-Infrared	5310 B	TOC Analyzer
Dissolved Organic Carbon	Combustion-Infrared	5310 B	TOC Analyzer
UV-absorbance at 254 nm	Spectrophotometry	5910 B	

[1]Reference: Standard Methods for the Analysis of Water and Wastewater, 20th Edition, 1998

4.7 Cryptosporidium Preparation, Enumeration, and Viability Methods.

4.7.1 Oocyst Preparation.

Prior to each experiment, the Wisconsin State Laboratory of Hygiene prepared an oocyst stock suspension. Oocysts were supplied to the State Lab of Hygiene by two sources. Oocysts used in experiments performed between April 3 and July 27, 2000 were supplied by Pat Mason at Pleasant Hill Farms in Troy, Idaho. These oocysts were harvested from infected calf feces and purified by discontinuous sucrose gradient centrifugation. All other experiments received oocysts from Marilyn Marshall at the University of Arizona in Tucson, Arizona. These oocysts were harvested from calf feces and were purified using a sucrose gradient followed by a cesium chloride gradient. Both sources of oocysts were of the Iowa strain. Prior to enumeration procedures, each batch of oocysts was microscopically screened

to ensure no bacterial contamination was present and to conduct a general assessment of the oocyst batch.

4.7.2 Enumeration Methods

Flow Cytometry-Cell Sorting (FCCS) was used to enumerate the number of oocysts. Figure 4d depicts the steps used in the enumeration protocol described in Appendix E.
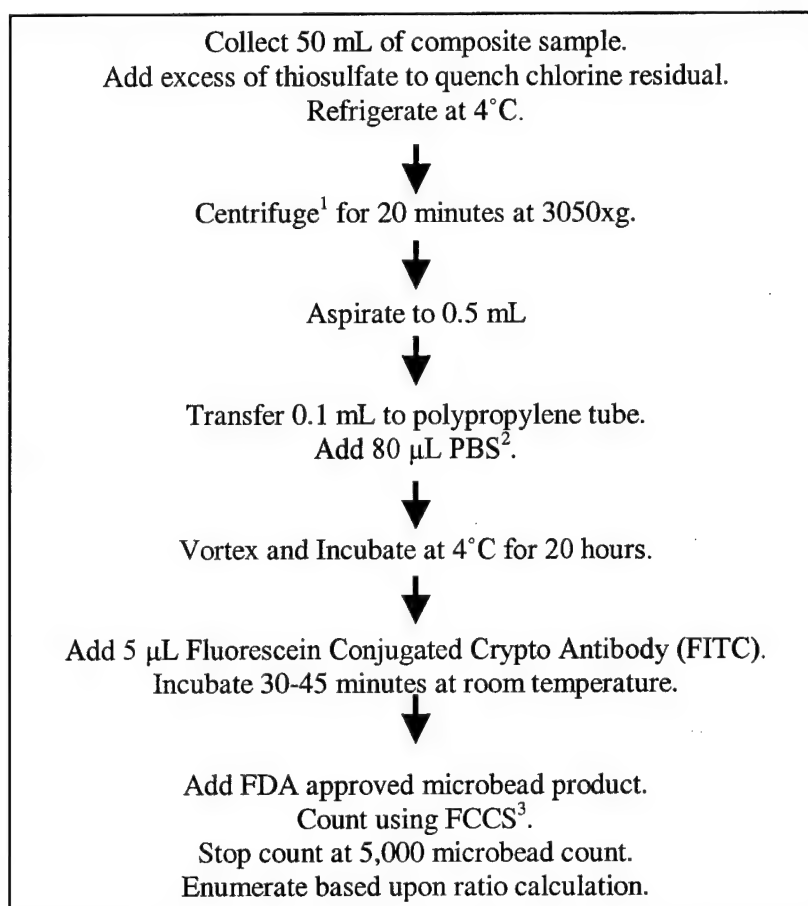
Collect 50 mL of composite sample.
Add excess of thiosulfate to quench chlorine residual.
Refrigerate at 4°C.

Centrifuge[1] for 20 minutes at 3050xg.

Aspirate to 0.5 mL

Transfer 0.1 mL to polypropylene tube.
Add 80 μL PBS[2].

Vortex and Incubate at 4°C for 20 hours.

Add 5 μL Fluorescein Conjugated Crypto Antibody (FITC).
Incubate 30-45 minutes at room temperature.

Add FDA approved microbead product.
Count using FCCS[3].
Stop count at 5,000 microbead count.
Enumerate based upon ratio calculation.

[1]BeckmanSBC Centrifuge
[2]PBS = phosphate buffered saline (5% goat serum/0.01% Tween 20)
[3]EPICS Elite FCCS, Beckman-Coulter Miami, FL

Figure 4d FCCS Enumeration Procedure
(Adapted from R.M. Hoffman, 2000)

4.7.3 Viability Methods

Flow Cytometry-Cell Sorting (FCCS) was used to assess the number of viable oocysts. Figure 4e depicts the enumeration protocol described in Appendix E.

Three different antibodies were used in evaluating the viability. Cy5 and Texas Red were used to identify an individual particle as *C. parvum* or not (red particles are positive). Cy5 is detectable by the FCCS at 633 nm generated by the HeNe laser. The Texas Red was used for visibility in the red range for confirmation by ocular techniques (Cy5 requires special optical attachments for microscopic interrogation). SYTO 9 was used to assess viability by measuring green fluorescence at 488 nm as generated by the Argon laser. The FCCS generates a histogram that groups the number of particles meeting the above-specified "gating" criteria. These data were then compared to control samples prepared by heat inactivation.
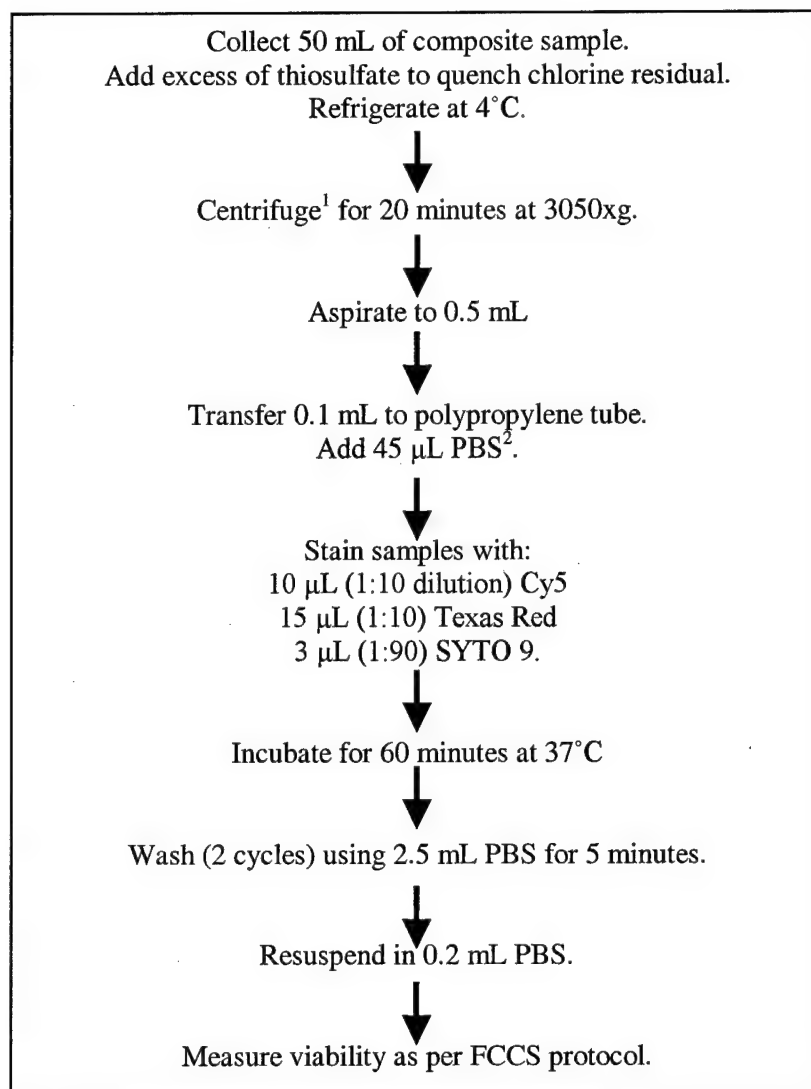
Collect 50 mL of composite sample.
Add excess of thiosulfate to quench chlorine residual.
Refrigerate at 4°C.

↓

Centrifuge[1] for 20 minutes at 3050xg.

↓

Aspirate to 0.5 mL

↓

Transfer 0.1 mL to polypropylene tube.
Add 45 μL PBS[2].

↓

Stain samples with:
10 μL (1:10 dilution) Cy5
15 μL (1:10) Texas Red
3 μL (1:90) SYTO 9.

↓

Incubate for 60 minutes at 37°C

↓

Wash (2 cycles) using 2.5 mL PBS for 5 minutes.

↓

Resuspend in 0.2 mL PBS.

↓

Measure viability as per FCCS protocol.

[1]BeckmanSBC Centrifuge
[2]PBS = phosphate buffered saline (5% goat serum/0.01% Tween 20)
[3]EPICS Elite FCCS, Beckman-Coulter Miami, FL

Figure 4e FCCS Viability Procedure
(Adapted from R.M. Hoffman, 2000)

4.8 Tracer Study

Several step-dose tracer studies were conducted to determine the hydraulic characteristics of each reactor. Sodium chloride (Fisher Scientific, Pittsburgh, PA) was used as a tracer and was fed through the influent chlorine tubing. Samples were collected from each reactor effluent and conductivity was measured using a conductance meter (YSI Model 35, Yellow

Springs Instrument Co., Inc., Yellow Springs, OH) equipped with a YSI Model 3403 conductivity cell. p-Nitrophenol (Fisher Scientific, Pittsburgh, PA) was also used as a tracer and was fed through the influent chlorine tubing. Samples were collected from each reactor effluent and absorbance was measured using a spectrophotometer (Spectronic® 20, Genysis™).

Three different reactor volumes were analyzed in duplicate to verify experimental repeatability. Two different tracers, sodium chloride and p-Nitrophenol, were used to ensure the selected tracers met the criteria required of tracers. The reactor conditions established in these tracer studies are listed in Table 4.5 to 4.7. Due to results obtained in earlier studies, some additional studies were conducted to focus on improving the mixing conditions in the 500 mL reactors (Currey, 2001). The only parameter that was changed was the use of a 5 cm long teflon stir bar rather than a 2 cm long teflon stir bar.

Table 4.5
Tracer Study Reactor Conditions with NaCl as Tracer

Reactor	Volume (mL)	Flow Rate (mL/min)	$\tau_{nominal}$ (min)
1	2250	25.8	87.2
5	2250	25.7	87.6
2	1230	21.2	57.9
6	1230	20.3	60.5
3	580	18.3	31.7
7	580	18.1	32.1

Note: 500 mL reactors used a 2 cm long stir bar
         1230 mL and 2250 mL reactors used a 10 cm long stir bar

Table 4.6
Tracer Study Reactor Conditions for 500 mL Reactors with NaCl as Tracer

Reactor	Volume (mL)	Flow Rate (mL/min)	$\tau_{nominal}$ (min)
1	580	18	32.3
2	580	16.8	34.5

Note: 500 mL reactors used a 5 cm long stir bar.

Table 4.7
Tracer Study Reactor Conditions for 500 mL Reactors with p-Nitrophenol as Tracer

Reactor	Volume (mL)	Flow Rate (mL/min)	$\tau_{nominal}$ (min)
1	580	18	32.2
2	580	19	30.5

Note: 500 mL reactors used a 5-cm long stir bar

The first tracer study was performed to characterize the reactors. Additional tracer studies were performed to better characterize the 500 mL reactors with longer stir bars. Two different tracers, sodium chloride and p-Nitrophenol, were used to ensure the results and performance indices of the reactor were independent of the tracer selected. As demonstrated above, all tracer studies were conducted under very similar reactor conditions.

4.9 Data Evaluation Procedures

4.9.1 Reactor Hydraulics

A step-dose tracer study was used to characterize the hydraulics of the system. Residence time distributions were determined from F-curves generated for each reactor. Various performance indices were calculated from the ratios of percentile residence times. Characteristics of reactors of the same volume were evaluated by their average, standard deviation, and 95% confidence intervals. Equation 4.1 defines the 95% confidence interval.

$$\mu = X \pm t \times \frac{s}{n^{0.5}} \qquad (4.1)$$

where: μ = % confidence interval
X = mean value
s = standard deviation
t = number of degrees of freedom, 95% confidence
n = number of data points

The step-dose tracer results were evaluated by the CMFTRs in series model (Equation 3.28) using nonlinear regression. Nonlinear regression estimates for C_0, Z, and τ_{model} were computed for various flow rates and detention times.

4.9.2 Oocyst Removal and Inactivation.

Log removal and log inactivation were calculated from flow Cytometry results using Equations 4.2 and 4.3, respectively.

$$Log \, Removal = -\log\left(\frac{N}{N_0}\right) \tag{4.2}$$

where: N = concentration of infectious and noninfectious oocysts in chlorinated water
N_0 = concentration of infectious and noninfectious oocysts in feed water

$$Log \, Inactivation = -\log\left(\frac{N}{N_0}\right) \tag{4.3}$$

where: N = concentration of infectious oocysts in treated water
N_0 = concentration of infectious oocysts in feed water

4.9.3 CT Values.

CT values were calculated using Equation 3.32. Disinfectant concentration was calculated as the average chlorine concentration in the reactor effluent after the reactor had reached steady-state as defined in the tracer analysis. The nominal detention times were used as the t values.

4.9.4 Chlorine Kinetics.

Decay constants (k_d) were calculated for each experimental run, both disinfection experiments and inactivation experiments, using the rate laws shown in Equation 3.15 to 3.17. The method of least squares was used to calculate the decay constants. These were evaluated based upon coefficients of determination (r^2) for each decay model.

$$r^2 = \frac{SSY - SSE}{SSY} \tag{4.4}$$

where: SSY = sum of squared error with $y = \bar{y}$ as the model
SSE = sum of squared errors with the model being tested

$$SSY = \sum_{i=1}^{n} (y_i - \overline{y})^2 \tag{4.5}$$

where: n = number of observations
y_i = observed value
$\overline{y}$ = average of observed values

$$SSE = \sum_{i=1}^{n} (y_i - \hat{y}_i)^2 \tag{4.6}$$

where: $\hat{y}$ = values predicted by the proposed model

Section 5
Results & Discussion

5.1 Water Quality Analysis

Untreated (raw) Lake Mendota water and filtered effluent from the UW-CEE Pilot Water Treatment Plant were used in all experiments. A detailed description of the treatment processes involved in the pilot-scale water treatment facility is discussed in Section 4. Table 5.1 provides a summary of the eleven water quality parameters measured for the raw and filtered waters that were used in the *Cryptosporidium* feed tank. Because the source is a natural surface water, these values were presented as a range to describe the variability due to natural climate and environmental cycles.

Table 5.1
Lake Mendota Water Quality from February 2000 to May 2001

Parameter	Units	Raw Water		Filtered Water	
		Range	Median	Range	Median
pH	pH units	7.6 – 8.7	7.9	5.9 – 6.6	6.2
Turbidity	NTU	2.7 – 3.4	3.1	0.13 – 0.39	0.23
Total Hardness	mg/L as $CaCO_3$	290 – 430	365	270 – 430	410
Ca^{2+} Hardness	mg/L as $CaCO_3$	120 – 200	160	120 – 210	180
Mg^{2+} Hardness	mg/L as $CaCO_3$	170 – 230	205	150 – 240	210
Alkalinity	mg/L as $CaCO_3$	110 – 258	228	20 – 173	96
Ammonia	mg/L as N	0.1 – 0.3	0.2	0.1 – 0.3	0.2
TOC	mg/L	4.12 – 5.31	4.84	2.35 – 3.07	3.00
DOC	mg/L	4.18 – 5.33	5.22	2.92 – 3.59	2.99
UV_{254}	cm^{-1}	0.059 – 0.078	0.069	0.013 – 0.068	0.037
SUVA	L/mg-cm	0.011 – 0.015	0.013	0.004 – 0.023	0.015

Note: Water quality data are only reported for the waters used in *C. parvum* experiments.
5 samples were used for raw water
7 samples were used for filtered water
SUVA = Specific UV Absorbance = UV_{254}/DOC

Lake Mendota water can be characterized as very hard and well buffered. The pilot-scale WTP removes approximately 60% of the alkalinity present in the raw water. Ammonia was measured prior to both inactivation and disinfection experiments and ranged between 0.1 to 0.3 mg/L as N. This created a chlorine demand of approximately 0.8 to 2.3 mg/L as Cl_2. The TOC and DOC were larger for the raw water than the filtered water, as expected.

5.2 Reactor Hydraulics

5.2.1 Residence Time Distribution

The residence time distributions of the reactors were evaluated with respect to the targeted nominal detention times. The 2250 mL reactors, 1230 mL reactors, and 580 mL reactors were targeted to achieve detention times of 90, 60, and 30 minutes, respectively. F-curves were generated for each reactor to determine specific percentile residence times, including t_{10}, t_{50}, t_{90}, t_{95}, and the mean residence times. The average (AVG), standard deviation (STDEV), and 95% confidence interval (95% CI) for the various detention times were calculated for each set of reactor conditions. The results are listed in Tables 5.2 to 5.6. Detailed data for Tables 5.2 to 5.4 are presented in Currey (2001). Detailed data for Tables 5.5 and 5.6 are presented in Appendix G.

Table 5.2
Percentile Residence Times for 2250 mL Reactors

Parameter (min)	Reactor 1	Reactor 2	AVG	STDEV	95% CI
t_{10}	12.6	12.6	12.6	0.00	12.6 ± 0.00
t_{50}	62.6	61.4	62.0	0.85	62.0 ± 1.37
mean t	84.8	80.6	82.7	2.92	82.7 ± 4.7
$\tau_{nominal}$	87.2	87.6	87.4	0.28	87.4 ± 0.5
t_{90}	212	220	216	5.66	216 ± 9.16
t_{95}	272	244	258	19.8	258 ± 32.1

Table 5.3
Percentile Residence Times for 1230 mL Reactors

Parameter (min)	Reactor 1	Reactor 2	AVG	STDEV	95% CI
t_{10}	9.50	10.2	9.85	0.50	9.9 ± 0.80
t_{50}	48.1	47.1	47.6	0.71	47.6 ± 1.15
mean t	67.8	64.3	66.1	2.45	66.1 ± 4.00
$\tau_{nominal}$	57.9	60.5	59.2	1.85	59.2 ± 2.99
t_{90}	148	144	146	2.83	146 ± 4.58
t_{95}	636	660	648	17.0	648 ± 27.5

Table 5.4
Percentile Residence Times for 580 mL Reactors with 2-cm Stir Bar

Parameter (min)	Reactor 1	Reactor 2	AVG	STDEV	95% CI
t_{10}	8.26	8.26	8.26	0.00	8.3 ± 0.00
t_{50}	30.4	30.3	30.4	0.07	30.4 ± 0.11
mean t	48.6	51.1	49.8	1.78	49.8 ± 2.88
$\tau_{nominal}$	31.7	32.1	31.9	0.24	31.9 ± 0.39
t_{90}	74.6	72.2	73.4	1.70	73.4 ± 2.75
t_{95}	444	432	438	8.49	438 ± 13.7

Table 5.5
Percentile Residence Times for 580 mL Reactors with 5-cm Stir Bar

Parameter (min)	Reactor 1	Reactor 2	AVG	STDEV	95% CI
t_{10}	4.5	3.9	4.2	0.41	4.2 ± 0.66
t_{50}	37.7	23.9	30.8	9.73	30.8 ± 15.8
mean t	39.3	32.6	35.9	4.68	35.9 ± 7.57
$\tau_{nominal}$	32.3	34.5	33.4	1.54	33.4 ± 2.49
t_{90}	50.1	94.8	72.4	31.59	72.4 ± 51.15
t_{95}	109	144	127	24.43	127 ± 39.56

Note: Sodium chloride was used as the tracer

Table 5.6
Percentile Residence Times for 580 mL Reactors with 5-cm Stir Bar

Parameter (min)	Reactor 1	Reactor 2	AVG	STDEV	95% CI
t_{10}	4.8	5.1	5.0	0.20	5.0 ± 0.33
t_{50}	37.9	28.1	33.0	6.93	33.0 ± 11.22
mean t	39.3	39.3	39.3	0.01	39.3 ± 0.02
$\tau_{nominal}$	32.2	30.5	31.4	1.20	31.4 ± 1.94
t_{90}	173	100.5	136.8	51.28	136.8 ± 83.04
t_{95}	182.6	120.7	151.7	43.82	151.7 ± 70.96

Note: p-Nitrophenol was used as the tracer

Often, the time to reach steady-state is assumed to be three times the nominal detention time for most flow-through systems. Table 5.7 describes the time to reach steady-state as defined by the ratio of $t_{90}/\tau_{nominal}$. With the exception of the 580 mL reactor with the 5-cm stir bar and the p-nitrophenol tracer, 3 times the nominal detention time was adequate to

reach steady-state based upon t_{90} data. Based upon analysis of all reactors using the 95% CI, it may be necessary to allow 6.8 times the nominal detention time to reach the 90th percentile values. However, it is important to note that in the experiments conducted for the 580 mL reactor with the 5-cm stir bar, sampling was not initiated from these reactors until 12 times the nominal detention time.

Table 5.7
$t_{90}/\tau_{nominal}$ as an Indicator for the Time to Reach Steady-State

Reactor Volume	AVG	STDEV	95% CI
2250	2.5	0.07	2.5 ± 0.11
1230	2.5	0.14	2.5 ± 0.23
580[1]	2.4	0.07	2.4 ± 0.11
580[2]	2.2	0.78	2.2 ± 1.3
580[3]	4.4	1.5	4.4 ± 2.4

Notes: [1] 580 mL reactor with 2-cm stir bar and NaCl tracer
[2] 580 mL reactor with 5-cm stir bar and NaCl tracer
[3] 580 mL reactor with 5-cm stir bar and p-nitrophenol tracer

Table 5.8 describes the time to reach steady-state as defined by the ratio of $t_{95}/\tau_{nominal}$. Based upon analysis of all reactors using the 95% CI, more than 3 times the nominal detention time would be required to reach the 95th percentile values. The focus of this analysis was upon the 2250 mL reactors because these reactors had the longest nominal detention time. From this analysis, it may be necessary to allow 3.3 times the nominal detention time of the 2250 mL reactor before sampling of any reactor can be initiated. Sampling was conducted in all experiments at approximately 4 times the nominal detention time of the 2250 mL reactor.

Table 5.8

$t_{95}/\tau_{nominal}$ as an Indicator for the Time to Reach Steady-State

Reactor Volume	AVG	STDEV	95% CI
2250	3.0	0.21	3.0 ± 0.34
1230	3.5	0.14	3.5 ± 0.23
580^1	2.7	0.14	2.7 ± 0.23
580^2	4.5	0.35	4.5 ± 0.57
580^3	4.9	1.2	4.9 ± 2.0

Notes: [1]580 mL reactor with 2-cm stir bar and NaCl tracer
[2]580 mL reactor with 5-cm stir bar and NaCl tracer
[3]580 mL reactor with 5-cm stir bar and p-nitrophenol tracer

Further validation of this analysis was conducted using chlorine and no *Cryptosporidium* to verify that steady-state would be obtained. Results of these experiments suggest that 3 x $\tau_{nominal}$ would be sufficient for chlorine residuals to stabilize within $\pm$ 0.3 mg/L (Currey, 2001). This was observed in all *C. parvum* experiments.

5.2.2 CMFTRs in Series Model

Nonlinear regression was used to fit the tracer study data with the CMFTRs in series model. The estimated values of Z and τ_{model} can be seen in Table 5.9. Values of Z equal to 1 were obtained for all reactors, indicating that the reactor behaved close to an ideal CMFTR.

Table 5.9

Percentile Residence Times for All Reactors

Parameter	Z	$\tau_{model}/\tau_{nominal}$ (min/min)	$\tau_{model}/\tau_{nominal}$ Range
2250	1	1.05	0.93 - 1.20
1230	1	1.12	0.97 - 1.32
580^1	1	1.34	1.14 - 1.61
580^2	1	1.07	0.93 - 1.22
580^3	1	1.31	1.23 - 1.40

Notes: [1]580 mL3reactor with 2-cm stir bar and NaCl tracer
[2]580 mL reactor with 5-cm stir bar and NaCl tracer
[3]580 mL reactor with 5-cm stir bar and p-nitrophenol tracer

The values of τ_{model} were compared to the nominal hydraulic residence time and values greater than 1.0 for the ratio of $\tau_{model}/\tau_{nominal}$ were obtained for most of the reactors. This suggests water is spending more time in the reactors than is predicted by $\tau_{nominal}$, and, therefore some fluid is spending time in a stagnant zone within the reactors.

The variance of both the flow rate and the volume of the reactors may affect the true value of the nominal detention time. Together, the variances of the two parameters were used to determine the variance of $\tau_{nominal}$. This variance resulted in a range of values listed in Table 5.9. The true detention time lies somewhere in this range. This range suggests that the 2250 mL and 1230 mL reactors are behaving more closely to ideal CMFTR conditions than the 580 mL reactors. The additional tracer studies with the 2-cm and 5-cm stir bars indicate that the longer stir bar assisted in making the 580 mL reactors behave more closely to a CMFTR.

5.2.3 Performance Indices

Other indices were calculated to evaluate the performance of the reactors. These indices are listed in Table 5.10. The mean $t/\tau_{nominal}$ and $t_{10}/\tau_{nominal}$ assist in quantifying the amount of fluid sitting in stagnant zones within the reactor rather than short-circuiting.

The Morril Dispersion Index (MDI) is defined as the t_{90}/t_{10} ratio. This index assists in evaluating the degree of mixing within the reactor. For an ideal CMFTR, the expected values of t_{10}/τ and t_{90}/τ are 0.105 and 2.303, respectively. This results in a MDI value of 21.9. Appendix G.3 presents the detailed calculations.

Comparison of the MDI obtained with the theoretical value shows that these reactors behaved close to ideal CMFTRs. The reactors did not reach ideal mixing conditions as evaluated by the MDI. For the 580 mL reactors, using a longer stir bar seemed to help improve mixing conditions.

Table 5.10
Residence Time Indices for All Reactors

Reactor Volume	Mean $t/\tau_{nominal}$	$t_{10}/\tau_{nominal}$	t_{90}/t_{10}
2250	1.04	0.145	17.2
1230	1.14	0.167	14.9
580[1]	1.40	0.259	8.9
580[2]	1.11	0.126	17.8
580[3]	1.52	0.159	27.8

Notes: [1]580 mL reactor with 2-cm stir bar and NaCl tracer
[2]580 mL reactor with 5-cm stir bar and NaCl tracer
[3]580 mL reactor with 5-cm stir bar and p-nitrophenol tracer
All values reported are the average of the two reactors sampled

5.3 Error Analysis

Nine experiments were performed with *Cryptosporidium parvum* throughout the course of this study. Seven of these experiments were conducted with *Cryptosporidium parvum* and chlorine to evaluate the effects of chlorination on inactivation. Two control experiments were run with *Cryptosporidium parvum* and no disinfectant in order to perform an error analysis on the sampling, analytical, and experimental procedures used in this study. The two control experiments were also used to ensure that no oocysts were lost in the system by settling or sticking to the materials (glass walls or rubber tubing) and that oocysts were not killed or excysted by the environmental stresses exerted from the system.

5.3.1 Sampling & Analytical Error

As noted earlier, samples were collected from the feed tanks at the beginning, midpoint, and end of each experiment to ensure that oocyst feed concentrations did not decrease due to settling. These samples were also analyzed for oocyst viability to ensure that oocyst die-off did not occur in the feed tank. Results shown in Tables 5.11 and 5.12 show no decrease in either the total number of oocysts or the number of infectious oocysts. Therefore, mixing was adequate to keep oocysts suspended and there was no die-off of viable oocysts.

Because there was no change in concentration, the feed samples can be used to assess the variability in collecting and analyzing samples. The sampling and analytical error was based on the standard deviation of the log concentration values attained from the feed tank samples.

Nine runs were analyzed for the total number of *Cryptosporidium parvum* oocysts, and all nine runs were used in the calculation of the sampling and analytical error. Analysis was also conducted on the live oocyst concentrations. The results can be seen in Tables 5.11 and 5.12. From this analysis, the log concentration did not vary more than ± 0.28 log units.

A control beaker was added in all experiments after 1 August 2000 to verify that oocysts did not die-off with time. This control beaker contained a 200 mL sample of the feed water. This beaker was sampled at the beginning and the end of the experiment. These results can be seen in Tables 5.13 and 5.14. Again, this confirmed that oocysts did not die-off through the course of the experiment because there was no decrease in the concentration of viable oocysts. The log concentration of oocysts did not vary more than ± 0.11 log units. Again, there was no difference when examining live oocyst concentration.

Table 5.11

Log Concentration of Total Oocysts at Various Times During Each Experiment
[log (total oocysts/L)]

Experiment	Feed 1[1]	Feed 2[2]	Feed 3[3]	Average Log Concentration	Variance
3 April 2000	5.42	5.52	5.38	5.44	0.005
22 April 2000	6.40	6.55	6.42	6.46	0.007
7 May 2000	5.77	5.70	5.75	5.74	0.001
1 August 2000	6.10	6.13	6.18	6.14	0.002
12 September 2000	5.90	5.83	6.07	5.93	0.015
20 September 2000	5.90	5.96	5.94	5.93	0.001
26 September 2000	6.19	6.08	6.03	6.10	0.007
24 February 2001	6.05	6.16	6.23	6.15	0.008
7 April 2001	6.03	6.03	6.01	6.02	0.000
				Average Variance	0.005
				Standard Deviation	0.28

Note: [1]Feed 1 corresponds to the sample at the beginning of the experiment
[2]Feed 2 corresponds to the sample at the midpoint of the experiment
[3]Feed 3 corresponds to the sample at the end of the experiment

Table 5.12
Log Concentration of Live Oocysts at Various Times During Each Experiment
[log (live oocysts/L)]

Experiment	Feed 1[1]	Feed 2[2]	Feed 3[3]	Average Log Concentration	Variance
3 April 2000	5.42	5.51	5.38	5.44	0.005
22 April 2000	6.33	6.46	6.37	6.39	0.004
7 May 2000	5.75	5.67	5.71	5.71	0.002
1 August 2000	6.05	6.07	6.10	6.07	0.001
12 September 2000	5.85	5.78	6.02	5.91	0.016
20 September 2000	5.88	5.94	5.92	5.91	0.001
26 September 2000	6.16	6.04	6.00	6.07	0.007
24 February 2001	6.03	6.13	6.21	6.12	0.008
7 April 2001	5.98	5.97	5.95	5.97	0.000
				Average Variance	0.005
				Standard Deviation	0.27

Note: [1]Feed 1 corresponds to the sample at the beginning of the experiment
[2]Feed 2 corresponds to the sample at the midpoint of the experiment
[3]Feed 3 corresponds to the sample at the end of the experiment

Table 5.13
Log Concentration of Total Oocysts at Various Times
During Each Experiment in the Control Beaker
[log (total oocysts/L)]

Experiment	Control 1[1]	Control 2[2]	Average Log Concentration	Variance
1 August 2000	6.65	6.69	6.67	0.000
26 September 2000	7.41	7.28	7.35	0.009
24 February 2001	7.99	8.20	8.10	0.022
7 April 2001	7.37	7.27	7.32	0.005
			Average Variance	0.009
			Standard Deviation	0.10

Note: [1]Control 1 corresponds to the sample at the beginning of the experiment
[2]Control 2 corresponds to the sample at the end of the experiment

Table 5.14
Log Concentration of Live Oocysts at Various Times
During Each Experiment in the Control Beaker
[log (live oocysts/L)]

Experiment	Control 1[1]	Control 2[2]	Average Log Concentration	Variance
1 August 2000	5.57	5.51	5.54	0.002
26 September 2000	8.00	7.85	7.93	0.011
24 February 2001	8.57	8.80	8.69	0.027
7 April 2001	7.95	7.85	7.90	0.005
			Average Variance	0.011
			Standard Deviation	0.11

5.3.2 Experimental Error

The experimental error of the methods used in this study includes variability in system parameters like flow rates and reactor volumes as well as the sampling and analytical error. The experimental error was calculated from the results of the control experiments performed on 3 April and 12 September 2000. The samples were analyzed according to the nominal detention time of the various reactors. The experimental error was found to be ±0.32 log units (see Tables 5.15 and 5.16).

Table 5.15
3 April 2000 Experimental Error [log (total oocysts/L)]

Reactor Volume	Reactor 1	Reactor 2	Reactor 3	Reactor 4	Variance
2250 mL	6.03	5.48	5.73	5.26	0.109
1230 mL	5.14	6.06	5.48	NA	0.219
580 mL	5.75	6.02	5.67	NA	0.033
				Average	0.120
				Standard Deviation	0.32

Note: NA = Not Analyzed

Table 5.16
12 September 2000 Experimental Error [log (total oocysts/L)]

Reactor Volume	Reactor 1	Reactor 2	Reactor 3	Variance
2250 mL	6.17	5.99	5.38	0.038
1230 mL	6.05	6.45	6.11	0.045
580 mL	6.20	6.10	6.03	0.008
			Average	0.030
			Standard Deviation	0.16

5.3.3 Log Removal and Log Inactivation

Log removal was calculated from the results of the control experiments performed on 3 April and 12 September 2000. This was performed to ensure that oocysts were not lost in the system by settling in the reactors or by sticking to the tubing or the reactor walls. Log removal calculations were based on the change in the concentration of total oocysts. As demonstrated by Table 5.17, the results showed that oocysts were not lost in the system because removals were within the experimental error of ±0.32 log units. Table 5.18 indicates that there was no inactivation exerted from the experimental conditions. Therefore, any log inactivation observed in all other experiments can be solely attributed to the effect of chlorine.

Table 5.17
Log Removal Results

Experiment	Range	Median	Average	Standard Deviation
3 April 2000	-0.62 to 0.31	-0.26	-0.22	0.32
12 September 2000	-0.52 to -0.06	-0.18	-0.23	0.16

Table 5.18
Log Inactivation Results

Experiment	Range	Median	Average	Standard Deviation
3 April 2000	0.00 to 0.02	0.01	0.01	0.00
12 September 2000	0.03 to 0.07	0.04	0.04	0.02

To summarize, mass balances on the reactors were performed for each experiment. The mass balance indicated a sampling and analytical error of ±0.28 log units and an

experimental error of ±0.32 log units. Log removal was analyzed for each experiment and indicated that no oocysts were lost due to settling or sticking to the system materials. Log removal was analyzed for each experiment and indicated that there was no oocyst die off due to the experimental set up.

5.4 Chlorination at Low CT Values.

Experiments were performed between April and 1 August 2000 with *Cryptosporidium parvum* and chlorine at CT values typical of those found in municipal water treatment facilities. These experiments were performed at pH 6.0. Currey (2001) presented a detailed analysis of these experiments and Tables 5.19 through 5.21 provide a summary of this analysis.

Table 5.19
Experiment Summary (April to 1 August 2000)

Experiment Date	Water Type
22 April	Filtered
7 May	Raw
28 June[1]	Filtered
27 July[1]	Filtered
1 August	Raw

Notes: [1]Enumeration and viability could not be performed due to low numbers of oocysts in the samples.

Table 5.20
Log Removal Results

Date	CT (mg-min/L)	AVG	STDEV	Range
22 April 2000	53 to 779	0.00	0.13	-0.25 to 0.17
7 May 2000	103 to 633	0.13	0.13	-0.03 to 0.28
1 August 2000	63 to 345	0.08	0.12	-0.04 to 0.37

Table 5.21
Log Inactivation Results

Date	CT (mg-min/L)	AVG	STDEV	Range
22 April 2000	53 to 779	0.20	0.10	0.05 to 0.33
7 May 2000	103 to 633	0.05	0.02	0.02 to 0.07
1 August 2000	63 to 345	0.07	0.02	0.05 to 0.10

As demonstrated in Table 5.20, there was not a loss of oocysts from settling or sticking to the reactor walls. The log removal calculated was within the same standard deviation for all three experiments. As shown in Figure 5a, there was no trend observed between log inactivation and CT values. Chlorine was ineffective for inactivating *Cryptosporidium parvum* at pH 6.0 and CT values less than 779 mg-min/L for both raw and treated Lake Mendota water. In effect, there was no log inactivation occurring when these data are analyzed with respect to the previously stated experimental error.
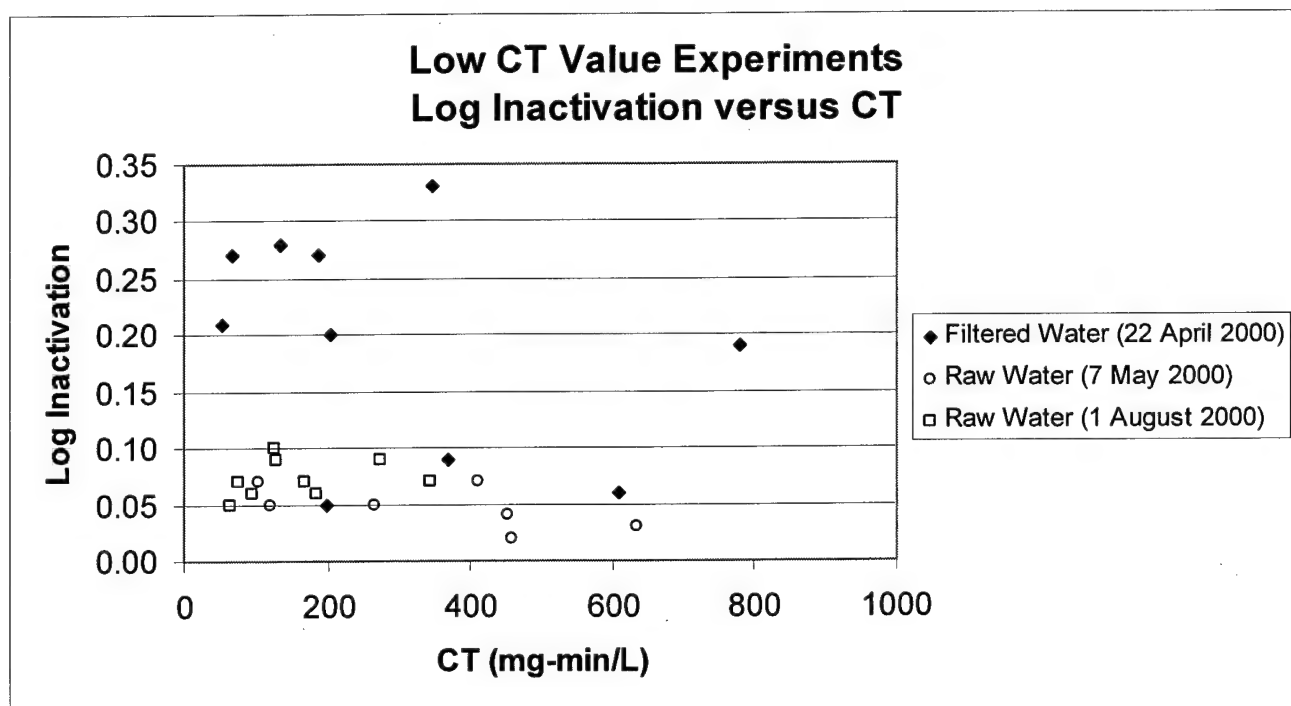
Figure 5a Summary of log inactivation at low CT values

5.5 Chlorination at High CT Values

5.5.1 High CT Value Chlorination Conditions

Because no inactivation of *Cryptosporidium parvum* was seen under CT conditions typical of those found in current practice, the study evaluated inactivation at higher CT values. Experimental conditions required to achieve these increased CT values demonstrated some operational problems that impaired viability assessment. For example, under different operating conditions of detention time and chlorine concentration, analysis was prohibited

due to the formation of precipitates in both the reactors and the samples.

The original concept to gain increased CT values was to maintain the nominal detention times and solely vary the chlorine concentration. Chlorine concentrations in excess of 20,000 mg/L were analyzed in September 2000. This presented two problems. First, the pH of the reactor could not be maintained at pH 6.0 due to an increase in the pH of the chlorine feed tank. Attempts to decrease the chlorine feed tank pH by buffering (phosphate buffer system) or acid adjustment resulted in a depletion of any free available chlorine after a short duration of time, approximately 4 hours. In addition to the pH problem, dechlorination with sodium thiosulfate caused a yellow precipitate to form in the sample vials. This was not observed when sodium bisulfite was used as the dechlorination agent. This is detailed by Currey (2001) and was demonstrated to be a function of CT value.

This approach to just increase the chlorine concentration was abandoned. Adjusting both the nominal detention time and chlorine concentration to achieve a target CT value range of 3,000 mg-min/L to 20,000 mg-min/L was implemented. Under this new approach, nominal detention times targeted 60, 120, and 180 minutes for the 580 mL, 1230 mL, and 2250 mL reactors, respectively. Experiments were performed with *Cryptosporidium parvum* and chlorine at the new reactor conditions between February and April 2001.

For these experiments, the chlorine stock solutions were targeted at 5,000 mg/L, 8,000 mg/L, and 12,000 mg/L. Again, the chlorine stock solution pH was very high, approximately pH 11.6 to 12.0. This chlorine feed tank condition resulted in a steady-state reactor pH of approximately 7.5, with a range typically from 7 to 8. Further attempts to buffer the chlorine stock solution were performed. A 0.1M phosphate buffer was found to maintain a very stable chlorine solution for more than 36 hours at pH 9. However, this resulted in the formation of a white precipitate in the reactors within one hour of addition of the chlorine to the reactor. Using a phosphate buffer pH control strategy was abandoned and 0.1M bicarbonate was added to decrease the chlorine stock solution pH to approximately 9. Feed tank pH was adjusted from pH 6.0, as performed in all previous experiments, to pH 4.3. This eliminated the formation of the white precipitate, but did not result in a final reactor pH of 6.0. The reactor pH at steady-state still ranged from pH 7 to 8.

5.5.2 High CT Chlorination Analysis

Experiments were performed between September 2000 and April 2001 with *Cryptosporidium parvum* and chlorine at increased CT values. These experiments targeted a final reactor pH equal to 6.0. As noted above, this target pH was never obtained. Table 5.22 shows the steady-state pH values at which the samples were taken.

Table 5.22
Experiment Summary (September 2000 to April 2001)

Experiment Date	Water Type	Chlorine Feed Tank	pH
20 September 2000[1]	Filtered	• 8180 to 67400 mg/L • unbuffered • final pH 11.6 to 12	8 to 9
26 September 2000[1]	Raw	• 10680 to 74200 mg/L • unbuffered • final pH 11.6 to 12	8 to 9
24 February 2001	Raw	• 5539 to 12984 mg/L • unbuffered • final pH 11.6 to 12	7.2 to 8.7
3 March 2001[2]	Filtered	• 5103 to 12445 mg/L • phosphate buffer • final pH 8.54 to 8.82	7.4 to 8.1
7 April 2001	Filtered	• 4579 to 12408 mg/L • 0.1M HCO_3^- addition • final pH 8.85 to 9.02	7.8 to 8.6

Notes: [1]Viability could not be assessed in several reactors due to yellow precipitate.
[2]Viability could not be assessed due to formation of white precipitate.

Log removal and log inactivation analysis are summarized in Tables 5.23 and 5.24, respectively. Log inactivation was ineffective above the pK_a of 7.5 and at pH values equal to $pK_a \pm 0.30$. Log inactivation for raw and filtered water are depicted in Figures 5b and 5c, respectively. Analysis of the experiments conducted with raw water does indicate an increase in log inactivation with an increase in CT value. However, the log inactivation achieved is generally not outside the experimental error. The opposite trend seems to occur in the experiments conducted with filtered water. From this, chlorine was ineffective in inactivating *Cryptosporidium parvum* at pH 7.2 to 9.0 with CT values greater than 2000 mg-min/L for both raw and treated Lake Mendota water.

Table 5.23
Log Removal Results

Date	CT (mg-min/L)	AVG	STDEV	Range
20 September 2000	3528 to 39528	0.24	0.10	0.16 to 0.35
26 September 2000	3766 to 16337	0.05	0.01	0.03 to 0.06
24 February 2001	3234 to 17720	-0.15	0.13	-0.36 to 0.06
7 April 2001	2102 to 23123	-0.08	0.43	-0.75 to 0.51

Table 5.24
Log Inactivation Results

Date	CT (mg-min/L)	AVG	STDEV	Range
20 September 2000	3528 to 39528	0.32	0.093	0.25 to 0.43
26 September 2000	3766 to 16337	0.07	0.014	0.06 to 0.08
24 February 2001	3234 to 17720	0.25	0.17	0.14 to 0.62
7 April 2001	2102 to 23123	0.25	0.18	0.08 to 0.48
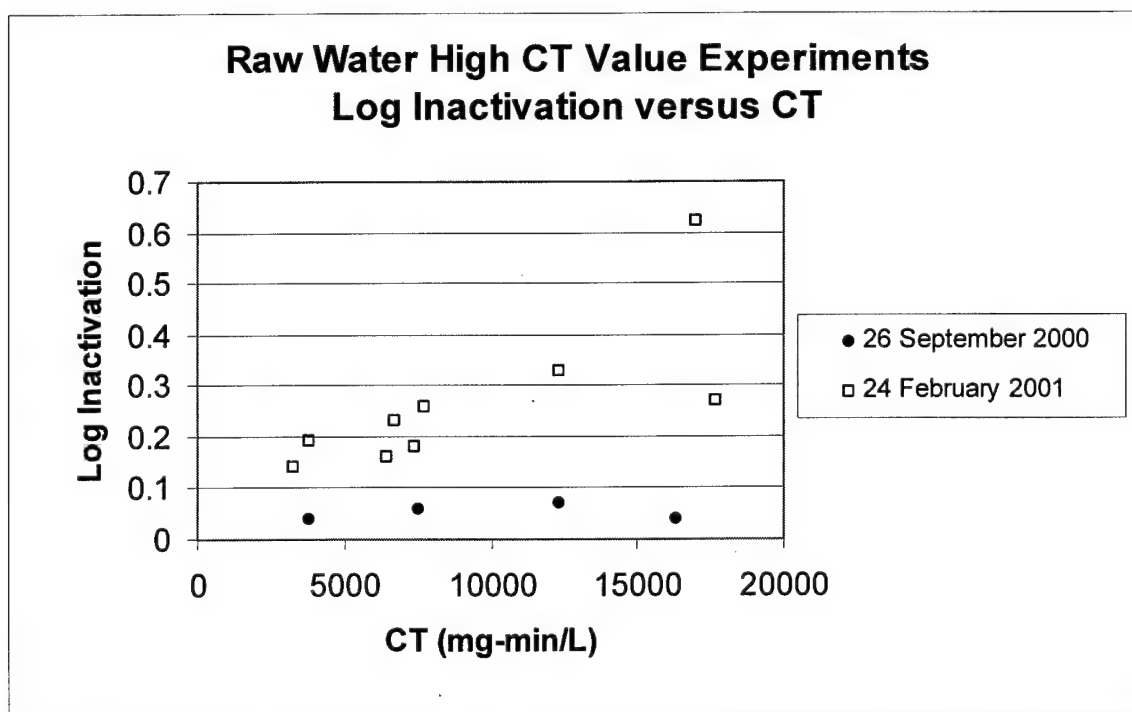
Figure 5b Log inactivation data for raw lake water at high CT values
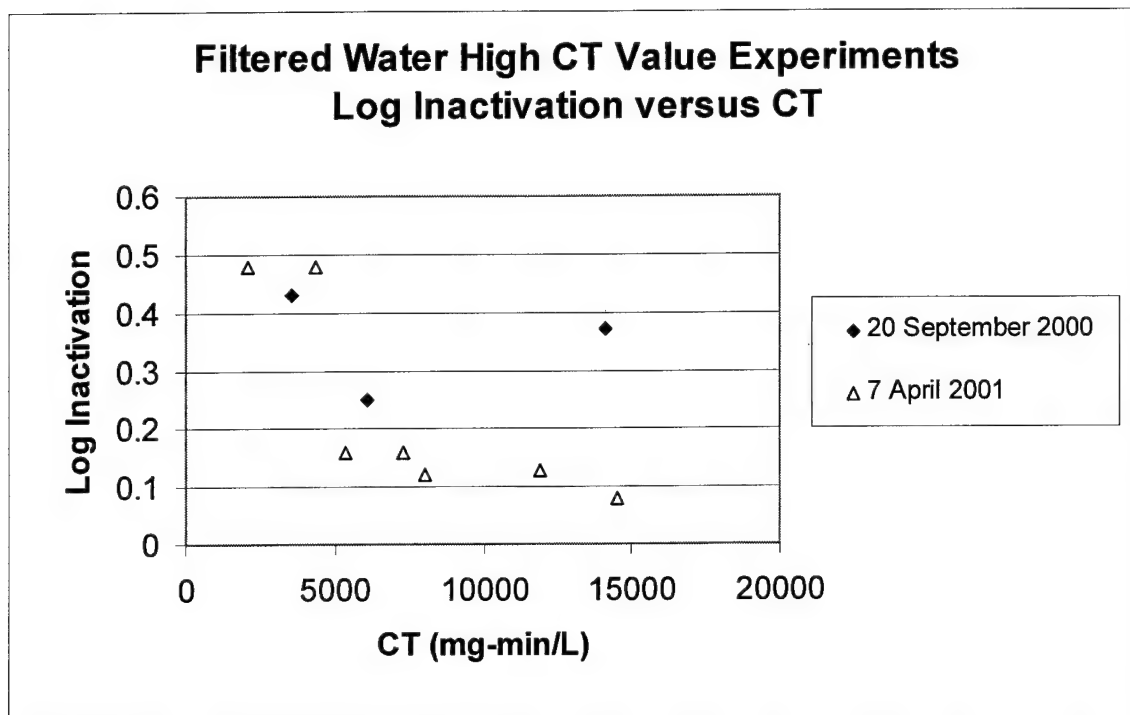
Figure 5c Log inactivation data for filtered lake water at high CT values

5.6 Chlorine Decay Kinetics

5.6.1 Chlorine Decay Kinetics for Inactivation Experiments

Natural waters contain numerous chlorine demanding substances. In order to accurately model and predict inactivation in these waters, characterization of the chlorine demand must be evaluated. Simple first and second-order decay models, Equations 3.15 and 3.16, were examined to determine the reaction order that best describes chlorine decay. These decay constants (k_d) were calculated for each experiment and the k_d calculated for each of the inactivation experiments previously discussed are presented in Tables 5.25 and 5.26. The nonlinear least squares regression method was used to estimate the decay constants and r-squared values (r^2).

Table 5.25

Chlorine Decay Constant Analysis for Inactivation Experiments with Raw Water

Date	Order	k_d	r^2
7 May 2000	1	6.0×10^{-3}	0.66
	2	1.1×10^{-3}	0.69
1 August 2000	1	3.4×10^{-2}	0.27
	2	1.2×10^{-2}	0.29
26 September 2000	1	3.0×10^{-5}	0.99
	2	1.0×10^{-8}	0.86
24 February 2001	1	1.7×10^{-3}	0.91
	2	1.9×10^{-5}	0.91

Table 5.26

Chlorine Decay Constant Analysis for Inactivation Experiments with Filtered Water

Date	Order	k_d	r^2
22 April 2000	1	4.5×10^{-3}	0.40
	2	8.2×10^{-4}	0.41
20 September 2000	1	6.0×10^{-4}	0.98
	2	1.3×10^{-5}	0.94
7 April 2001	1	1.5×10^{-3}	0.01
	2	1.5×10^{-5}	0.92

Decay constants for raw water are expected to be higher than those for filtered water based upon the raw water containing more disinfectant demanding substances. This was generally observed and can be seen by comparing the data in Tables 5.25 and 5.26. The r^2 values indicate that, in most cases, there is little difference in the ability of either model to describe the system.

5.6.2 Chlorine Decay Kinetics for Low Detention Time CMFTR Experiments

One of the primary concerns in the different models to accurately differentiate between the different orders of reaction might be the lack of data at smaller detention times. The system was run using smaller detention times to better differentiate between first and second-order predictions. These experiments were conducted with chlorine disinfectant only and no *Cryptosporidium* oocysts. The smallest detention time that could be obtained was 3 minutes. Experiments were conducted for both raw and filtered water and are presented in

Table 5.27. Detailed analysis of these experiments is presented in Appendix I.3 and I.4. As shown in Table 5.27, the r^2 values indicate that there is little difference in the ability of either model to describe the system.

Table 5.27
Chlorine Decay Constant Analysis for Low Detention Times

Water Type	Order	k_d	r^2
Raw	1	4.6×10^{-2}	0.98
	2	9.2×10^{-3}	0.99
Filtered	1	3.9×10^{-2}	0.94
	2	7.5×10^{-3}	0.95

Note: Both experiments were conducted on 24 March 2001

5.6.3 Chlorine Decay Kinetics for Batch Experiments

As previously mentioned, the smallest detention time achieved was 3 minutes and this was the smallest detention time we could obtain with our experimental set up. Also, there was no discernible difference between the two models for fitting chlorine demand in the reactors. In order to evaluate the chlorine demand at a detention time smaller than 3 minutes, a batch experiment was conducted.

The purpose of the batch experiment was to develop a better estimate of chlorine decay at smaller detention times. A batch experiment was conducted for both raw and filtered water. The data and procedure used are presented in detail in Appendix I.5. The simple 1st order, simple 2nd order, second order overall models, Equations 3.15 to 3.17, were used to estimate k_d.

Table 5.28
Chlorine Decay Constant Analysis for Batch Experiment

Water Type	Order	k_d	r^2
Raw	1	6.2×10^{-3}	0.65
	2	4.1×10^{-3}	0.77
	2nd Overall	2.4×10^{-2}	0.96
Filtered	1	6.3×10^{-3}	0.974
	2	3.1×10^{-3}	0.973
	2nd Overall	1.4×10^{-3}	0.976

Note: Both experiments were conducted on 29 April 2001

As shown in Table 5.28, the 2nd order overall model provided the best fit for the raw water. There was not a significant difference in the fits for the filtered water. This is depicted for raw and filtered water in Figures 5d and 5e, respectively. By comparing both figures, the chlorine is consumed more rapidly in the raw water than the filtered water at the smallest detention times. This region of increased consumption at the smaller detention times most significantly influences the model estimates.
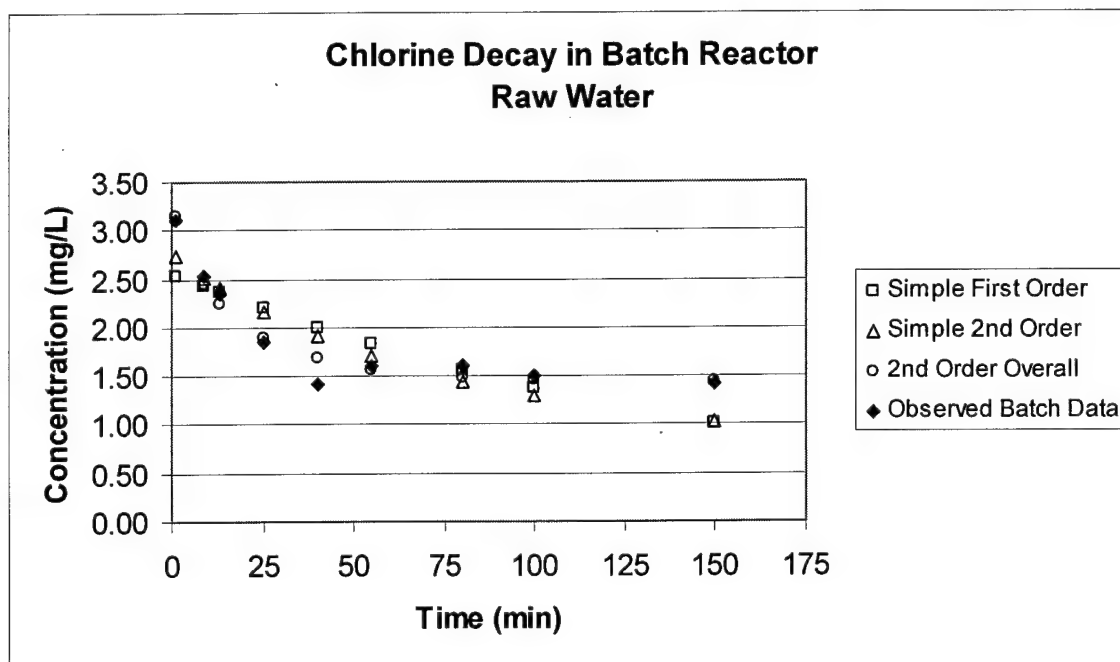
Figure 5d Raw water batch experiment chlorine decay
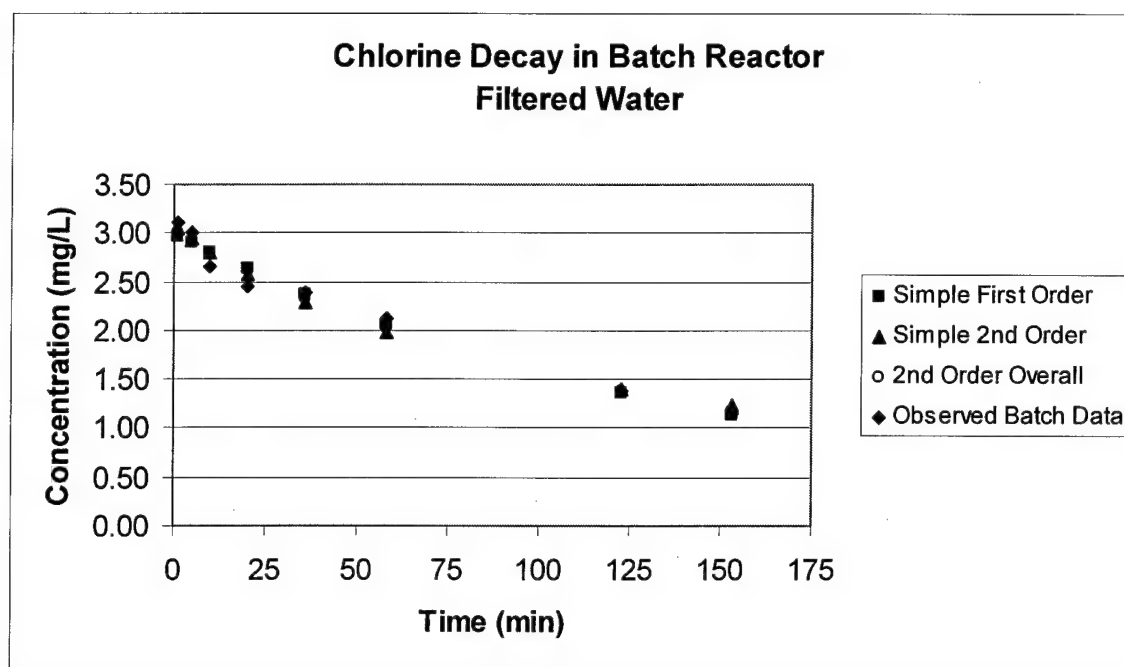
Figure 5e Filtered water batch experiment chlorine decay

To help design future experiments where k_d values can be accurately estimated from CMFTR data, the decay constants estimated from the batch experiments were then used to model predicted chlorine decay in a CMFTR. The CMFTR model predictions followed the same trend as those estimated by the batch experiment. Figures 5f and 5g show the CMFTR model predictions for raw and filtered water, respectively. Based on these results, it is recommended that future CMFTR experiments include reactors with nominal detention times smaller than 3 minutes to capture the rapid initial decay, particularly for the raw water. Also, future experiments should include reactors with nominal detention times longer than 180 minutes to describe the decay beyond the longest detention time of the inactivation experiment conditions.
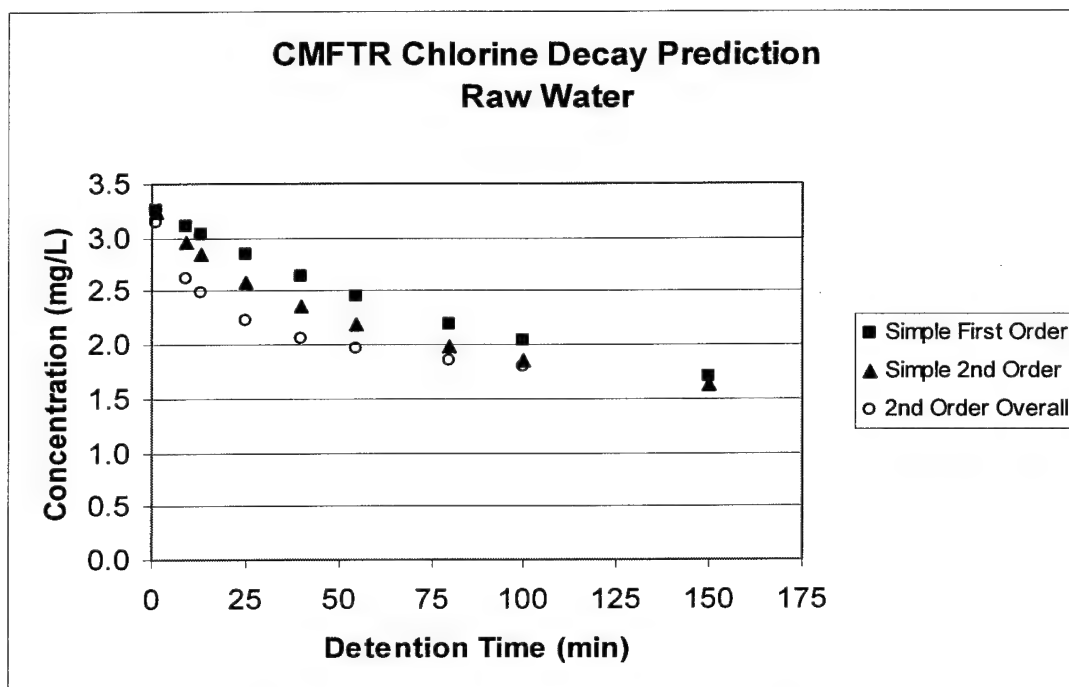
Figure 5f Raw water CMFTR chlorine decay modeling
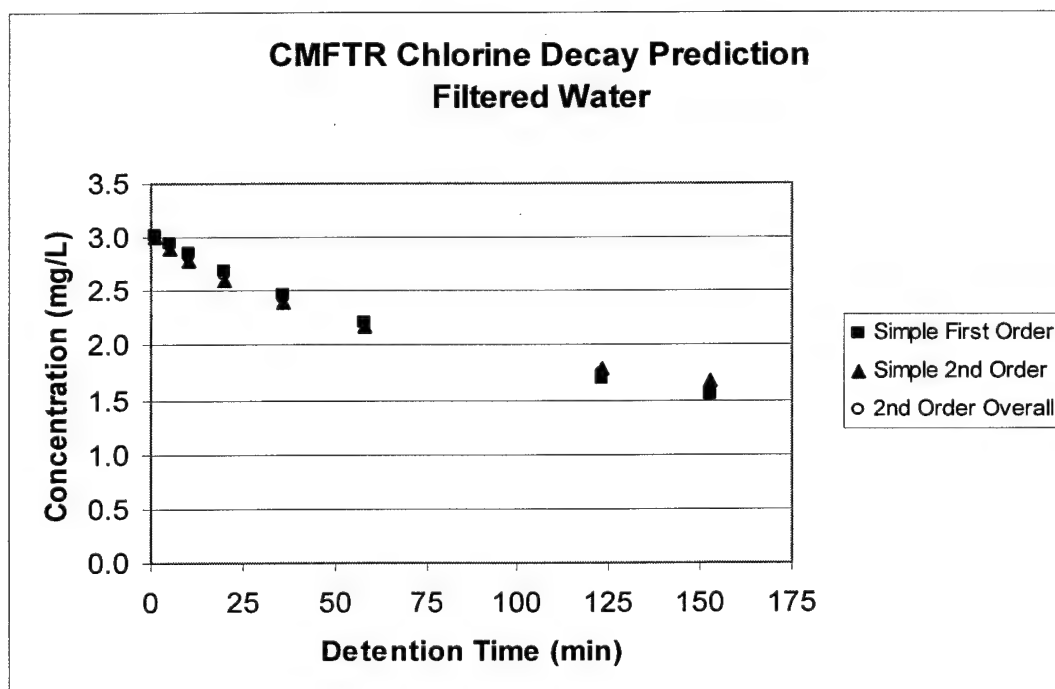
Figure 5g Filtered water CMFTR chlorine decay modeling

Section 6
Conclusions and Recommendations

This study analyzed the effects of chlorination on the inactivation of *Cryptosporidium parvum* in natural waters. Raw and filtered Lake Mendota feed waters were used to evaluate the influence of disinfectant demanding substances on the inactivation and the decay of chlorine within the system. Initially (February to August 2000), experiments were conducted to approximate inactivation at low CT values that would be representative of current chlorine disinfection practices in the United States. Additional experiments (September 2000 to April 2001) were conducted to evaluate effects at higher CT values required to obtain greater than 1-log inactivation as reported in the literature. The study utilized a system of CMFTRs to analyze these effects.

6.1 Conclusions

6.1.1 Chlorination of Cryptosporidium parvum

As reported by Currey (2001), at pH 6.0, chlorine was ineffective in inactivating *Cryptosporidium parvum* at CT conditions typically found in water treatment facilities in the United States. In this portion of the study, CT values ranged from 63 mg-min/L to 633 mg-min/L for raw water and 53 mg-min/L to 779 mg-min/L for filtered water. These CT conditions were inadequate to effect any quantifiable inactivation outside the established experimental error.

At pH 7.2 to 9.0, chlorine was ineffective at inactivating *Cryptosporidium parvum* at CT values ranging from 3,233 mg-min/L to 17,728 mg-min/L for raw water and from 2,103 mg-min/L to 39,521 mg-min/L for filtered water. Despite the use of several variations to obtain increased CT conditions, no inactivation was obtained with these increased CT values. Comparison of these results with published data suggests that inactivation is closely linked with system pH.

6.1.2 System Operation

Improvement of the system was evaluated by increasing the length of the magnetic stir bar used in 580 mL reactors and obtaining a reliable combination of nominal detention

time and chlorine feed concentration to effect changes in CT conditions. Increasing the length of the stir bar produced an increase in performance in the 580 mL reactors as evaluated by two additional tracer studies. Water spent a longer time in the 580 mL reactors than predicted by the CMFTR in series model and performance indices previously described in Section 5. Approximates of these reactors were still described as one CMFTR.

CT conditions were varied by solely increasing the chlorine feed concentration and by a combination of nominal detention times and chlorine feed tank concentrations. Solely increasing the chlorine feed concentration proved to be unreliable to maintain a target feed concentration for more than 12 hours. Chlorine feed solutions greater than 20,000 mg/L demonstrated a loss of free available chlorine in excess of 50% within 4 hours. Increasing the nominal detention time in each reactor (twice the detention time used in the low CT condition experiments) and increasing the chlorine feed from 5,000 mg/L to 12,000 mg/L were evaluated to change CT conditions. The chlorine feed solution was successfully buffered using a 0.1M phosphate buffer system. However, this produced an instantaneous white precipitate in the reactors. Adding a 0.1M sodium bicarbonate solution maintained a stable chlorine feed concentration for greater than 24 hours.

Throughout all these increased CT conditions, steady-state reactor pH was never controlled to the target value of pH 6.0. pH control was limited to adjusting the pH of the chlorine feed solution and the pH of the water feed tank. Chlorine feed tank pH for the 0.1M sodium bicarbonate addition decreased pH to approximately 9.0. Water feed tank pH was varied from 6.0 to 4.3. pH values measured for all inactivation experiments under these increased CT conditions produced pH values from 7.2 to 9.0. Recommendations for possible system improvement are described in Section 6.2.2.

6.1.3 Chlorine Decay Kinetics

Chlorine decay constants for raw water were larger than the chlorine decay constants for filtered water because the presence of disinfectant demanding substances caused the chlorine to decay faster. These decay constants were evaluated for a temperature range of 20 to 22°C and at decreased nominal detention times in a characteristic flow-through design as previously described. This was obtained by increasing the water flow rate to maximum

practical values for the pump, tubing, and pump cartridge conditions used in the laboratory. Implementing a batch test to evaluate disinfectant decay at the smallest practical time limit was also evaluated. Three models, simple 1st order, simple 2nd order, and 2nd order overall, were used to predict the k_d for the batch system. The 2nd order overall provided the best estimate for the raw water. There was no distinguishable difference between the models for the filtered water. Further application of these k_d values to the three models for a CMFTR predicted the same trend that 2nd order overall may best represent the raw water disinfectant demand and no one model provided a better estimate for the filtered water. No experiments were conducted under variable chlorine concentrations or temperature ranges.

6.1.4 Disinfection Kinetics

No inactivation beyond the established experimental error was observed in the experiments. Application of the previously described inactivation models could not be established from the data obtained in this study. The influences of disinfectant demanding substances on inactivation were unable to be evaluated.

6.2 Recommendations

6.2.1 Reactor Conditions

The reactors exhibited different mixing conditions based on reactor size. Evaluation of the stir-bar length and tracer used in the tracer studies was conducted for the 580 mL reactors. Mixing conditions as evaluated by reported performance indices increased. However, the water is still spending more time in the reactor than predicted by the nominal detention time. Further consideration to implementing stir-bars with a different design could be evaluated. This was not evaluated in this study based upon concerns of these designs potentially damaging the influent glass tubing in the reactor. The influent glass tubing was extended to the deepest practical depth without interfering with the action of the stir-bar. Different designed stir-bars to induce more mixing energy in the vertical direction may increase mixing conditions, but may require a decrease in the depth of the influent tubing and increase the potential for reactor short-circuiting.

6.2.2 pH Control

Current strategies to change CT conditions, i.e. increasing nominal detention time and chlorine feed concentration between 5,000 mg/L to 12,000 mg/L, should be maintained. However, the system should be reengineered to introduce a pH control chemical at a point close to the reactor influent. The proposed design to accomplish this is presented in Figure 6a. Implementing another flow rate into the system may complicate operation of experimental conditions. However, this pH control can be used to achieve a target reactor pH of 6.0 throughout the duration of the experiment. Experience of controlling the water feed tank pH for 13 experiments indicates that a small volume of concentrated sulfuric acid will be required. All other attempts to effect pH control of the system have been evaluated and have resulted in problems linked to effectively evaluating the viability of the samples.

1. *C. parvum* Feed
2. NaOCl Feed
3. Additional T-fitting
4. Acid Feed
5. Effluent
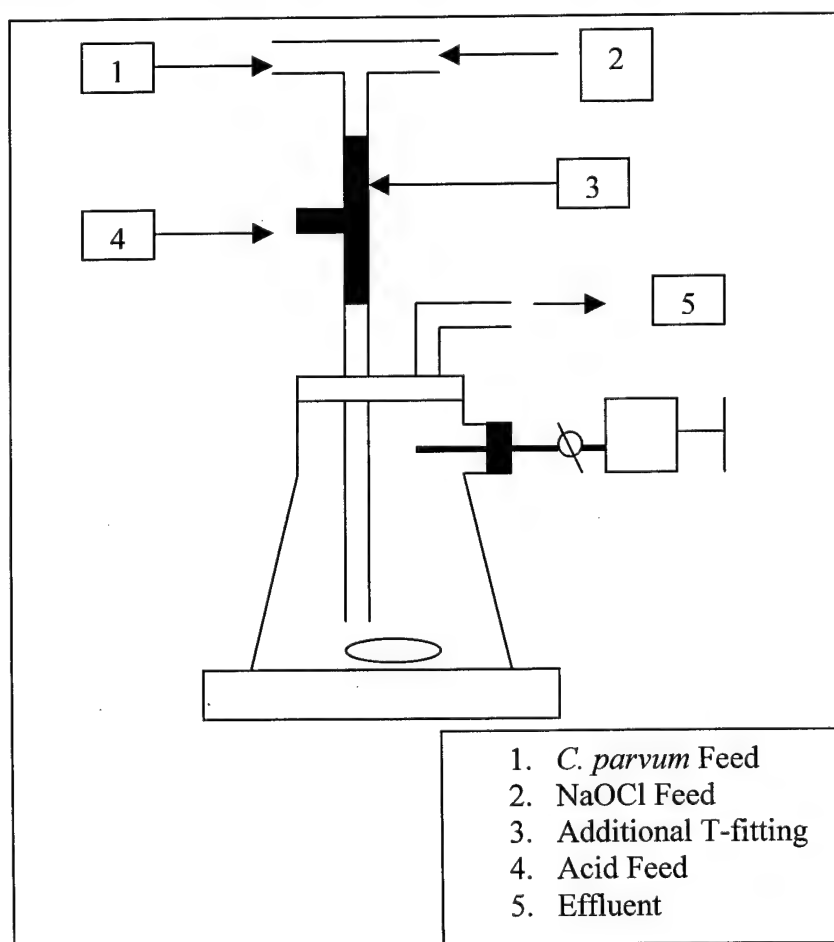
Figure 6a Proposed pH control design

6.2.3 Chlorine Decay Kinetics

Chlorine decay kinetic data used to approximate the disinfectant decay constant should be evaluated by both the decreased detention time in CMFTRs and the batch experiment protocol conducted in this study. This should be done over the course of a year to determine any influence of the natural lake cycles. The batch experiments should be expanded to produce a family of decay curves for different initial chlorine concentrations. Temperature effects should be evaluated for the range 4 to 6°C. These conditions should be applied to validate the specified decay constant ranges reported in this study.

6.2.4 Disinfection Kinetics

Inactivation data should be obtained for pH 6.0 at increased CT conditions. Once these data are acquired, the inactivation models presented in Section 3 should be evaluated to determine which model best describes the inactivation of *Cryptosporidium parvum* by chlorination. Once inactivation data are obtained, control runs should be done to validate the sampling, analytical, and experimental error. It will have been approximately one year since these data were collected and the system is being operated under different conditions by different researchers. Additional analysis can then be performed to evaluate the effect of pretreatment on the inactivation of the organism. Also, sensitivity analysis to determine the ability of the reactor system to inactivate *Cryptosporidium* should be performed once inactivation data is obtained.

Section 7
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Appendix A
Cryptosporidium parvum Lifecycle

Detailed lifecycle descriptions of C. parvum are well documented in the literature. The following summary and Figure are included to provide the technical definitions associated with each stage of the lifecycle.

Reproduction will occur upon excystation when the oocysts will release the sporozoite. Sporozoites that penetrate the cell walls of the intestinal wall are then referred to as a trophozoite or schizont. This attachment to the epithelial cells is achieved by an attachment organelle referred to as a "feeder organelle." The trophozoite or schizont will then undergo an asexual reproduction (multiple fission) producing 8 merozoites within the meront. These meronts will attach to the intestinal cell wall and are termed Type I meronts. Type I meronts are believed to continually reproduce indefinitely. However, a Type II meront is formed that releases 4 merozoites. Type II merozoites will then undergo sexual reproduction between the microgamete (male) and the macrogamete (female). A zygote is formed from this sexual reproductive stage. A resistant cell wall will form around a zygote, undergo meiosis, and form 4 sporozoites (this stage is termed sporogony). These oocysts are then shed into the environment in the feces of infected cattle. Figure A.1 depicts the lifecycle as described above.
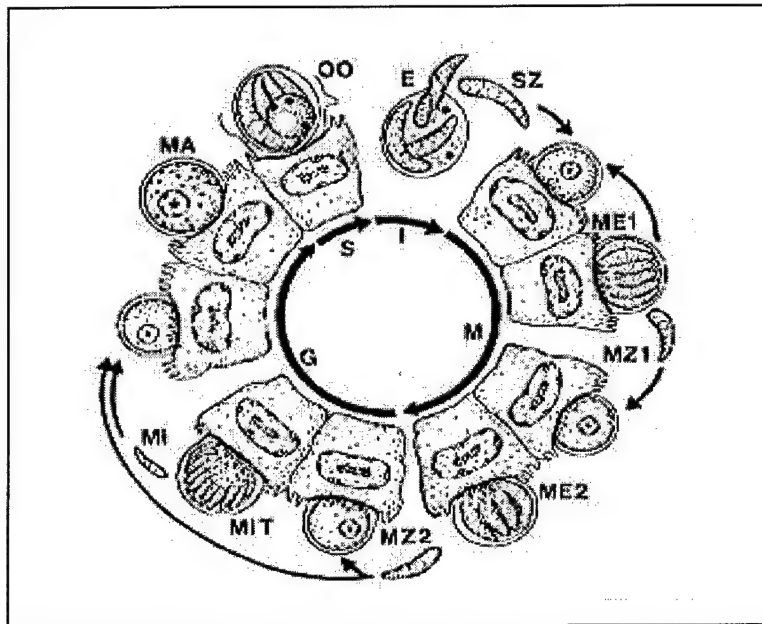
Figure A.1 *C. parvum* lifecycle.
(http://www.ksu.edu/parasitology/basicbiology.html with permission)

The abbreviations used are as follows:

- **(E)** Excystation (either as thick-walled oocysts from environment or via thin-walled oocysts excysting in situ), resulting in the release of 4 sporozoites through suture in wall.
- **(G)** Gamogony.
- Infective phase.
- **(M)** Merogony.
- **(ME1)** Type I meront containing 8 merozoites.
- **(ME2)** Type II meront 4 sporozoites.
- **(MA)** Macrogamete, containing wall forming bodies.
- **(Mi)** Microgamete.
- **(MiT)** Microgametocyte with 16 non-flagellated microgametes.
- **(MZ1)** Type I merozoite.
- **(MZ2)** Type II merozoite.
- **(OO)** oocyst.
- **(S)** Sporogony.
- **(SZ)** sporozoite.

(*Source*: Adapted from: http://www.ksu.edu/parasitology/basicbiology.html)

Appendix B
The Spectrum of Methods

A summary of the various techniques that may be possible for evaluation of environmental samples is presented in Tables B.1 and B.2. This is presented to describe how the data presented in the literature and the research has been derived. Inherent in all data is the variability and sensitivity of the methods used to evaluate the samples. A description of methods to evaluate the enumeration and viability of oocysts is presented in Table B.1. Advances in genetic genotyping and microbiological techniques have presented numerous opportunities of application into the identification of pathogenic microorganisms in environmental samples. The advantages and disadvantages presented in Table B.2 are relative with respect to the continued advances in this field. As progress continues, some of the disadvantages, such as trained analysts and availability of the equipment, may begin to dwindle.

Table B.1
Description of Reported Methods

Method	Description
Microscopy (DIC—Differential Interference Contrast)	Wet mount slide preparation and analysis under electron microscope.
Mouse Infectivity	Infects mice populations with various dose concentration and then analyze using necroscopy techniques.
Vital Dye Staining Acid-Fast Stain Modified Acid-Fast Stain Auramine-Rhodamine Stain	Uses chemicals (i.e. DAPI/PI or auramine-rhodamine) conjugates to stain. Modified protocol uses H_2SO_4 as the decolorizer.
EIA Diagnostic Test Kits FA Diagnostic Test Kits Immunofluorescence Assays (IFA--ASTM method)	Employs polyclonal and monoclonal antibodies (MAbs) to target a specific antigenic epitope that would fluoresce.
Enzyme-Linked Immunosorbent Assay (ELISA)	Uses enzyme techniques to target specific cells in conjunction with an in vitro technique.
USEPA Method 1622	Employs filtration, immunomagnetic separation (IMS), and fluorescence assay (FA) techniques. See Section 2.4.2 for explicit description.
USEPA Method 1623	Same as for Method 1622. Employs a Cryptosporidium-Giardia IMS kit.
FCCS	See Section 2.4.3 for explicit description.
Cell Culture	Use of tissue cells infected with sample under ideal growth conditions to grow the infectious agent.
In Vitro Excystation	In vitro simulation of the epigastric conditions (elevated temperature and acidic conditions in the presence of bile salts)
Polymerase Chain Reaction (PCR)	Use of a gel analysis and targets a specific DNA/RNA strand (18S rRNA gene) by using direct gene probes.

Table B.2
Comparison of Reported Methods

Method	Advantages	Disadvantages
Microscopy (DIC)	• Does not require concentration • Inexpensive	• Requires fluorescence microscope • Not sensitive to light infections
Mouse Infectivity	• Assesses the ability of the parasite to complete the lifecycle • Accepted as the "gold standard"	• Variety of mice are utilized • Mouse infectivity not correlated with human infectivity • Mice do not support growth of the Type 1 (H) genotype • Limited interlaboratory evaluation • Enumeration based upon excretion rates and math models may not be accurate to extrapolation for humans • Issues concerning animal rights for use in laboratory experiments
Acid-Fast Stain Modified Acid-Fast Stain	• Inexpensive • Can simultaneously detect other parasites	• May be less sensitive than some immunodiagnostic kits
Auramine-Rhodamine Stain	• Inexpensive • Rapid screening possible	• May be less sensitive than some immunodiagnostic kits • Requires fluorescence microscope
EIA Diagnostic Test Kits	• Visual Interpretation • Easy to Perform • Good Screening Technique • Easy to test large number of samples • Can be automated • Increased sensitivity	• Dilution may be required • Wash step critical to avoid false positives • May be difficult to interpret visually • Higher cost
FA Diagnostic Test Kits	• Short examination time • Some reagents can simultaneously detect Giardia cysts • Can batch test • Bright fluorescence; easy to read • Can be read quickly	• Recommended concentration is time consuming • Requires fluorescence microscope • Higher cost
IFA	• Reduces cross-reaction with nontarget organisms	• Tedious procedure • Requires high levels of technical expertise • Poor interlaboratory reproducibility • Poor sensitivity • Uses sample flotation which is a known source of organism loss
USEPA Method 1622	• MDL is low (4 oocyst/L) • No false-positive or –negatives have been reported • Fast and easy to perform	• MDL methodology is under review
USEPA Method 1623	• Same as for Method 1622 • Simultaneous detection of two parasites	• Same as for Method 1622 • Uncertainty of interferences from IMS kit

Method	Advantages	Disadvantages
FCCS	• Decreased processing time • Decreased costs • Greater sample volume analyzed	• Requires a FCCS
In Vitro Excystation	• Easy to use • Low cost • Resembles pathogenisis	• Requires incubation time • Experimental variability • Requires large numbers of oocysts
Cell Culture	• Implemented since 1970s in Cryptosporidium pharmaceutical research • High sensitivity to single oocysts has been reported	• Requires incubation time
PCR	• Specificity to a specific strand or genotype is possible	• Requires equipment and trained analysts

Appendix C
Regulatory Summary

A summary of the published regulatory guidelines within the Code of Federal Regulations (CFR) is presented. Two important revelations to note associated with *C. parvum* are the lack of any prescriptive regulations for the control of exposure to recreational waters and the absence of any log credit for disinfection practices using chlorine. Although this is a simplified summary, the regulations that have been promulgated and proposed represent the collaborative work of technical work groups assembled for that purpose. These work groups draw upon the leaders within the regulatory, research, and water utility communities.

C.1 United States Department of Agriculture (USDA) (7 CFR)

The USDA does not prescribe any regulatory guidance. However, the USDA does have administrative responsibilities over the Animal Paristology Institute located in Beltsville, Maryland. It is at this institute that government directed research is conducted that has been the basis for knowledge about the parasite from biological studies to disinfection of recreational waters.

C.2 United States Food and Drug Administration (FDA) (21CFR)

The FDA has not promulgated any standards within the food industry as it has for other parasites (i.e. *Toxoplasma gondii*). However, the Center for Food Safety and Applied Nutrition (CFSAN) does publish The Foodborne Pathogenic Microorganisms and Natural Toxins Handbook, (a.k.a The Bad Bug Book), which serves as a clearinghouse of information for food industry professionals. The FDA focus is upon the potential of fertilization of salad vegetables with manure as a potential source. Therefore, the 7th edition of the FDA's Bacteriological Analytical Manual contains a method for the examination of vegetables for *C. parvum*.

C.3 United States Occupational Safety and Health Administration (OSHA) (29CFR)

No reported control values or treatment practices. However, OSHA does establish BIOSAFETY procedure compliance in the workplace.

C.4 United States Environmental Protection Agency (40CFR)

C.4.1 Water Regulations

The EPA regulations that are applicable to the drinking water industry are the product of technical work groups addressing the trade-off of risks. The trade-off lies between the simultaneous control of microbial pathogens and the carcinogenic byproducts associated with the use of disinfectants, specifically chlorine. In order to address these risks and to provide "seamless" protection as these regulations are promulgated and implemented, the EPA has established the Microbial /Disinfection Byproducts (M-DBP) Rules. One subset of rules, known as the Disinfection and Disinfection Byproducts (D/DBPs) Rules, addresses the reduction of known and suspected carcinogenic byproducts from the use of chlorine disinfectant. The other subset is known as the Microbial Contamination Rules that focus upon inactivation/removal of a specified list of known waterborne pathogens (specified are viruses, *Giardia lamblia*, and *C. parvum* while other pathogens are under a review on Microbial Contaminant Candidate List).

Major milestones of the drinking water regulations are presented in Table C.1. Detailed emphasis is placed upon the Interim Enhanced Surface Water Treatment Rule (IESWTR), Information Collection Rule (ICR), Long Term 1 Enhanced Surface Water Treatment and Filter Backwash Rule (LT1FBR), and the Stage 2 M-DBP Agreement in Principle (AIP). These rules are in the compliance, data gathering or public review/comment phase of final promulgation. It is these rules that are shaping the future of the drinking water industry and technology and require research generated data to assist in making clear and reliable decisions.

The future direction of the regulations is dependent upon the protozoa data set that is being compiled under the ICR. The ICR has created much controversy within the water industry as to defining and refining the method of detection to an acceptable standardized level that resulted in the implementation of the ICR Supplemental Survey (ICRSS) to ensure a statistically viable and representative data set was obtained. Using FCCS generated spike samples coupled with a stringent sampling and chain-of-custody protocol (outlined in

Connell et al, 2000) and IMS separation procedures added (from ICR to ICRSS), a more reliable data set was obtained. Data analyzed across the 40 utilities indicates a statistically reliable data set. The important data analysis to consider is that only 15% of the utilities had a mean *Cryptosporidium* concentration greater than 0.075 oocysts per liter and none had a concentration greater than 0.5 oocysts per liter. Assessing this data into the development of the Bin Requirements outlined in the Stage 2 M-DBP AIP and described in Table C.2, only 15% of the utilities would be required to demonstrate a 1-log treatment.

Table C.1
Drinking Water Regulations Summary

Regulation	Summary	Key Dates
Safe Drinking Water Act (SDWA)	• Established MCL for a list of organic and inorganic compounds.	1974
Total Trihalomethane (TTHM) Rule	• MCL of 0.100 mg/L	1979
SDWA	• Established the requirement for maximum contaminant level goals (MCLG).	1986 Amendments
Surface Water Treatment Rule (SWTR)	• MCLG of zero for Giardia lamblia, viruses, and Legionella for surface water or GWUDI[1]. • Requires disinfectant residual in distribution system. • Removal and/or inactivation of 3-log for Giardia and 4-log for viruses. • Combined filter effluent of 0.5 NTU (95%) and 5 NTU (max) based upon 4-hour monitoring.	1989
Total Coliform Rule (TCR)	• MCL for TC of 5% positive (40 samples per month) or no more than one positive sample (less than 40 samples per month). • If positive sample is detected, must test for fecal coliforms or E. coli.	1989
SDWA	• Established the requirements for the National Primary Drinking Water Regulation (NPDWR). • Specifies either a MCL or treatment technique. • Requires public comment and cost analysis. • Regulations to address filter backwash. • Requires disinfection "as necessary" for ground water systems. • Established the timeline for Stage 1 DBPR, IESWTR, Stage 2 DBPR, and LTESWTR[2]	1996 Amendments
Information Collection Rule (ICR)	• Requires PWS greater than 100,000 to report on chemical byproducts and pathogens (Cryptosporidium, Giardia, and viruses) for 18 months. • PWS must conduct treatment studies to evaluate DBP precursor removal and DBP control.	1996
IESWTR, Final Rule	• Applicable for PWS serving more than 10,000 people. • MCLG of zero for Cryptosporidium. • 2-log Cryptosporidium removal for systems that filter. • Combined filter effluent of 0.3 NTU (95%) and 1 NTU (max) based upon 4-hour monitoring. • Individual filter effluent of 0.5 NTU after first 4-hours and 1 NTU (max) based upon two consecutive 15 minute monitoring must be reported to the state on a monthly basis. • Added Cryptosporidium to the definition of GWUDI. • Required to cover all treated water storage facilities. • Estimated $307 million for implementation. • Deadline: December 2001.	1999

Regulation	Summary	Key Dates
Stage 1 D/DBP Rule	• Applicable for all PWS that are CWS or NTNCWs[3]. • Defines Maximum Residual Disinfectant Level Goals (MRDLG) and MRDL for chlorine, chloramines, and chlorine dioxide. • Defines Maximum Contaminant Level Goals (MCLG) and MCL for four trihalomethanes, two haloacetic acids, bromate, and chlorite. • Deadline: December 2001 (serve more than 10,000) and December 2003 (serve less than 10,000).	1999

LT1FBR, Proposed	• Applicable for PWS serving less than 10,000 people.
	 • Same provisions as specified in IESWTR, Final with the following differences:
	    o Disinfection benchmark provisions.
	    o Treatment technique in lieu of MCL.
	    o Recycle flows to be introduced prior to the point of primary coagulant addition.
	 • Estimated $97.5 million for implementation.
	 • Deadline: Comments received by 9 June 2000.
		April, 2000

Stage 2 M-DBP Agreement in Principle	• Outlines goals to be addressed in passing the Stage 2 DBP Rule and the LT2ESWT Rule with promulgation in 2002.
	 • LT2ESWTR specifics:
	    o Conduct C. parvum monitoring for 24 months.
	    o Exemptions for 2.5 logs of treatment in addition to conventional treatment.
	    o "Toolbox" approach based upon source water monitoring with requirements specified in 4 "bins"
	    o Specific Log Credit based upon various treatment approaches (i.e. watershed control, alternative source, pretreatment, improved treatment, improved disinfection, or system performance validation.
	    o Stringent disinfection requirements for unfiltered systems.
	    o Specifications for uncovered finished reservoirs.
	    o Requirement for EPA to generate UV Light "IR" tables (analogous to CT tables for disinfectants).
		2000

Notes:
[1]GWUDI = ground water under the direct influence of surface water.
[2]LTESWTR = Long Term Enhanced Surface Water Treatment Rule.
[3]CWS = community water system and NTNCW = nontransient noncommunity water system.

Table C.2
Stage 2 M-DBP AIP Bin Requirements

Bin Number	Average Cryptosporidium Concentration (oocysts/L)	Additional Treatment Requirement
1	C. parvum < 0.075/L	No Action
2	0.075/L < C. parvum < 1.0/L	1-log treatment
3	1.0/L < C. parvum < 3.0/L	2.0 log treatment
4	C. parvum > 3.0/L	2.5 log treatment

Note: Adapted from USEPA FACA, 2000.

C.4.2 Air Regulations   No specified data found.

C.4.3 Solid Waste Regulations

40CFR503 addresses Land Application of materials.   Specifically, subpart D addresses Pathogens and Vector Attraction Reduction. It is in this section that the definitions and performance standards to classify sewage as a Class A or Class B are defined. There are many different protocols to characterize a Class A sewage and include fecal coliform test less than 1000 MPN (most probable number) and *Salomonella* less than 3 MPN per 4 grams of Total Solids (dry). *C. parvum* is not specified as a performance standard. However, analysis of land application materials as a possible pathway is highlighted.

C.5 United States Public Health Service (USPHS) (42CFR)

The USPHS does not publish any enforceable regulations for *C. parvum*. However, the do post health advisories, fact sheet, and preventive measure campaigns to help ensure widespread integration of promulgated regulations and prevention measures.

C.6 United States Department of Transportation (DOT) (49CFR)

DOT regulations are applicable when shipping known BIOHAZARDS and require specific chain-of-custody procedures to be followed.

Appendix D
Epidemiologic Summary

Epidemiological evidence has attributed outbreaks of cryptosporidiosis to the ingestion of contaminated drinking and recreational water. A variety of sources (surface water and groundwater) undergoing a variety of treatment processes (conventional, direct filtration, chlorination) were documented in drinking water scenarios.

D.1 Epidemiological Surveillance Techniques

One of the most difficult parts of waterborne cryptosporidiosis is how to detect an outbreak in a timely manner so that engineering controls can remediate the problem and take corrective action. In other words, how can an epidemiologic surveillance be conducted to decrease the impact and spread of disease? The CDC specifies six approaches to epidemiologic surveillance (CDC, 1997):

- Monitor sales of antidiarrheal medications.
- Monitor logs maintained by Health Maintenance Organizations (HMOs) and hospitals for complaints of diarrheal illness.
- Monitor incidence of diarrhea in nursing homes.
- Monitor laboratory data for *Cryptosporidium*.
- Monitor tap water in selected cities.
- Make immediate epidemiologic assistance available.

These approaches to surveillance should be of interest to environmental engineers not only as to the role the engineer will play in determining an epidemic source, but as a source of information to assist in monitoring a water supply.

D.2 Review of Documented Drinking Water Outbreaks

In a ten-year period (1984 – 1994), there were 10 documented outbreaks of waterborne cryptosporidiosis in the United States from drinking water sources. From 1995 to 1998, there have two documented outbreaks associated with drinking water. Both of these

resulted from fecal contamination entering the water source. WBDOs associated with contaminated drinking water are summarized in Table D.1.

Two outbreaks of cryptosporidiosis were not reported in Table D.1 because it is unclear as to their inclusion based upon the definition of a WBDO attributed to drinking water. These outbreaks were reported by Solo-Gabriele and Neumeister (1996), but were not reported by Craun et al (1998). Review of the CDC MMWR indicates that their inclusion is not appropriate.

- Bernalillo County (Albuquerque), New Mexico (1986). Seventy-eight confirmed cases of cryptosporidiosis were documented. However, it is unclear as to the source of the contamination amongst drinking water, swimming in contaminated surface water, or activities at a day care center.
- Alachua, Florida (1995). Seventy-two confirmed cases of cryptosporidiosis amongst attendees and counselors at a summer day camp. The source of the contamination was at the point of distribution where a hose in the vicinity of a garbage can cleaning area was used to fill water jugs.

D.3 Review of Documented Recreational Water Outbreaks

Contaminated recreational waters, such as community swimming pools, fountains, and streams/lakes, pose a major environmental pathway for waterborne cryptosporidiosis. A detailed summary of the documented outbreaks from 1988 to 1998 is presented in Table D.2. The analysis period was chosen based upon published data from the CDC-EPA WBDO surveillance system and to highlight the fact that although drinking water outbreaks have significantly declined, recreational waters still pose a significant risk.

Table D.1

Summary of Waterborne Cryptosporidiosis Associated with Drinking Water in the United States (1984 – 1998)

Location	Year	# Illness[1]	Water Source	Treatment[2,3,4]	Cause	Reference
Braun Station, TX	1984	117 (2,000)	Well	Cl_2	Sewage Contaminated Well	D'Antonio et al, 1989
Carrollton, GA	1987	(13,000)	River	Conv/Cl_2	Treatment Deficiencies	Hayes et al, 1989
Berks County, PA	1991	(551)	Well	Cl_2	GWUISW[5]	Moore et al, 1994
Medford, OR	1992	(3,000)	Spring/River	Filt/Cl_2	Treatment Deficiencies	Leland et al, 1993
Talent, OR	1992	Included Medford	Spring/River	Filt/Cl_2	Treatment Deficiencies	Leland et al, 1993
Milwaukee, WI	1993	(403,000)	Lake	Conv/Cl_2	Treatment Deficiencies	MacKenzie et al, 1994
Yakima, WA	1993	7	Well	None	Treat Deficiencies GWUISW	Craun et al, 1998
Cook County, MN	1993	27	Lake	Filt/Cl_2	Sewage Contamination	Craun et al, 1998
Clark County, NV	1994	103	River/Lake	Pre- Cl_2/Filt/Cl_2	Unknown	Roefer et al, 1995
Walla Walla, WA	1994	134	Well	None	Sewage Contamination	Craun et al, 1998
New Mexico	1998	9 (32)	Well	Cl_2	Nonpotable well not labeled	CDC, 2000
Texas	1998	89 (1400)	Well	Cl_2	Raw Sewage Spill	CDC, 2000
Totals:	*12*	*486 (422,983)*				

Notes:

[1](##) estimated number of sick.

[2]Conv = Conventional Treatment (Flash Mix/Flocculation/Sedimentation/Filtration).

[3]Cl_2 = Chlorine Disinfection.

[4]Filt = Filtration (Direct or In-Line Filtration).

[5]GWUISW = Groundwater Under the Influence of Surface Water.

Sources: Solo-Gabriele and Neumeister, 1996 and Craun et al, 1998 were used as precursory reference with actual references reviewed.

Table D.2

Summary of Waterborne Cryptosporidiosis Associated with Recreational Water in the United States (1988 – 1998)

Location	Year	# Illness[1]	Facility	Treatment[2]
Los Angeles, CA	1988	5 (44)	Pool	Cl_2
Idaho	1992	(500)	Water Slide	Cl_2
Oregon	1992	52(NA)	Pool (wave)	Cl_2
Wisconsin	1993	22(51)	Pool (motel)	Cl_2
Wisconsin	1993	(64)	Pool (motel)	Cl_2
Wisconsin	1993	(5)	Pool	Cl_2
Wisconsin	1993	(54)	Pool	Cl_2
Missouri	1994	26(101)	Pool (motel)	Cl_2
New Jersey	1994	46(2,070)	Lake	None
Kansas	1995	26(101)	Pool	NA
Georgia	1995	62(5,449)	Water Park	Cl_2
Nebraska	1995	14(NA)	Water Park	NA
Florida	1996	16(22)	Pool	NA
California	1996	29(3,000)	Water Park	Cl_2
Indiana	1996	(3)	Lake	None
Minnesota	1997	73(369)	Fountain	Sand Filter
Florida	1998	7	Day Care Pool	Cl_2
Minnesota	1998	7	Pool	Cl_2
Minnesota	1998	45	Swim Club	Cl_2
Oregon	1998	8(69)	Pool	Cl_2
Pennsylvania	1998	8	Lake	None
Wisconsin	1998	12	Pool	Cl_2
Wisconsin	1998	9	Pool	Cl_2
Wisconsin	1998	12	Pool	Cl_2
Totals:	*24*	*479(11,902)*		

Notes:

[1](##) estimated number of sick.

[2]Cl_2 = Chlorine Disinfection.

Sources: Carpenter et al, 1999 was used as precursory reference with actual CDC MMWR reports reviewed.

Appendix E
FCCS Sample Evaluation Protocol

The following describes the sampling protocol followed by the Wisconsin State Laboratory of Hygiene (SLH) for each sample. Rebecca M. Hoffman, Senior Microbiologist at the Wisconsin SLH, prepared this description.

E.1 Enumeration

50 mL samples were spun at 3050 x g for 20 minutes in a Beckman swinging bucket centrifuge. The supernatant was aspirated such that approximately 0.5 mL remained and 0.1 mL of each sample was transferred to a 12 x 75 mm polypropylene tube containing 80 µL phosphate buffered saline (pH 7.2) containing 5% goat serum (Sigma, St. Louis, MO) and 0.01% (v/v) Tween 20 (Sigma, St. Louis, MO). Samples were vortexed well and incubated at 4∘C overnight before incubation with 5 µL fluorescein conjugated anti-*Cryptosporidium* antibody (CryptaGlo, WaterBorne, Inc., New Orleans, LA). Oocysts were enumerated using flow cytometry and a FDA-approved microbead product (FlowCount, Beckman-Coulter, Miami, FL) originally designed for the enumeration of blood cells in human patients. An aliquot of this microbead product was added to the stained sample suspension. The sample was mixed and analyzed by flow cytometry (EPICS XL, Coulter Corporation, Miami, FL). Following enumeration of 5,000 microbeads, the sample flow was halted and the ratio of fluorescently labeled parasites to beads was multiplied by the lot-specific bead concentration factor to determine the number of parasites per µL sample.

E.2 Viability

0.1 mL of each sample was added to 12 x 75 mm polypropylene tubes containing 45 uL phosphate buffered saline (pH 7.2) containing 5% goat serum (Sigma, St. Louis, MO) and 0.01% (v/v) Tween 20 (Sigma, St. Louis, MO). Oocysts were stained with a 10 µL of a 1:10 diluted suspension of Cy5 conjugated antibody (CryptaGlo, WaterBorne, Inc., New Orleans, LA) to *Cryptosporidium* oocysts and 3 µL of a 1:90 diluted suspension of SYTO9 (Molecular Probes, Eugene, OR). Samples were incubated 60 minutes at 37∘C and washed twice with 2.5 mL phosphate buffered saline for 5 minutes. The samples were resuspended in 0.2 mL PBS prior to analysis by flow cytometry (EPICS Elite, Coulter Corporation, Miami, FL).

Cy5 positive events meeting the log side scatter and forward scatter criteria displayed by *Cryptosporidium* oocysts were evaluated for green (SYTO9) fluorescence. Positive and negative controls using live and heat inactivated oocysts respectively were stained for each run.

Appendix F
Chlorine Decay Model Derivations

Three models to estimate values of the disinfectant decay coefficient (k_d) for CMFTR are presented.

Mass Balance of the CMFTR:

$$0 = Q_0 C_0 + Q_D C_D - Q_f C_f - r_d V \qquad \text{(F.1)}$$

Assuming $C_0 = 0$

$$0 = Q_D C_D - Q_f Cf - r_d V \qquad \text{(F.2)}$$

F.1 Simple First Order

Assume $-r_d = -k_d C_f$

$$0 = Q_D C_D - Q_f Cf - k_d C_f V$$
$$Q_f C_f + k_d C_f V = Q_D C_D$$
$$C_f + k_d C_f \tau = \frac{Q_D C_D}{Q_f}$$

$$C_f = \frac{Q_D C_D}{Q_f (1 + + k_d \tau)} \qquad \text{(F.3)}$$

F.2 Simple Second Order

Assume $-r_d = -k_d C_f^2$

$$0 = Q_D C_D - Q_f C_f - k_d C_f^2 V$$
$$Q_f C_f + k_d C_f^2 V = Q_D C_D$$
$$k \tau C_f^2 + C_f = \frac{Q_D C_D}{Q_f}$$

Solve using quadratic equation.

$$C_f = \frac{1 - \sqrt{1 + 4 k_d \tau \, (\frac{Q_D C_D}{Q_f})}}{-2 k_d \tau} \qquad \text{(F.4)}$$

F.3 Second Order Overall

$$A + B \rightarrow \Pr oducts$$

Assume

$$\frac{dC}{dt} = -k_d C_A C_B$$

Solve using stoichiometry

$$-r_d = -k_d C_f^2 - kd(C_{B0} - C_{A0})C_f$$

(Note: Reference Harrington, 2000 CEE 822 Notes for full derivation of this relationship.)

$$0 = Q_D C_D - Q_f C_f - [k_d C_f^2 - k_d (C_{B0} - C_{A0})C_f]V$$

$$k_d C_f^2 V + k_d C_{B0} C_f V - k_d C_{A0} C_f V + Q_f C_f - Q_D C_D = 0$$

$$k_d C_f^2 \tau + k_d C_{B0} C_f \tau - k_d C_{A0} C_f \tau + C_f - C_{A0} = 0$$

Solve using quadratic equation.

$$C_f = \frac{(1 + k_d \tau C_B - k_d \tau C_A) - \sqrt{(1 + k_d \tau C_B - k_d \tau C_A)^2 + 4k_d \tau (C_A)}}{-2k_d \tau} \qquad (F.5)$$

Parameter	Units	R-5	R-6
Volume	L	0.58	0.58
Flow Rate	mL/min	18.0	16.8
$\tau_{nominal}$	min	32.3	34.5
Mean t (F-Curve)	min	39.2	35.0
Mean t (E-Curve)	min	39.3	34.3
Variance	min^2	1445	1025
t_{10}	min	4.5	3.9
t_{50}	min	37.7	24.1
t_{90}	min	50.1	95.3
t_{95}	min	152.9	100.6
$3*\tau_{nominal}$	min	96.8	103.4
Z		1	1
C_0	ms	3.05	2.66
τ_{model}	min	37.4	34.1
$\tau_{model}/\tau_{nominal}$	min/min	1.16	0.99
$(Variance_\tau)^2$	min^4	29.07	37.72
$Variance_\tau$	min^2	5.4	6.1
+Var Ratio		0.99	0.84
-Var Ratio		1.39	1.20
t_{90}/t_{10}	min/min	11.19	24.29
mean $t/\tau_{nominal}$	min/min	1.21	1.02
$t_{10}/\tau_{nominal}$	min/min	0.139	0.114

	R5	R6	AVG	STDEV	95% CI
$\tau_{nominal}$	32.3	34.5	33.4	1.537	2.489
Mean t (E-Curve)	39.3	34.3	36.8	3.521	5.701
t_{10}	4.5	3.9	4.2	0.388	0.629
t_{50}	37.7	24.1	30.9	9.624	15.583
t_{90}	50.1	95.3	72.7	31.986	51.794
t_{95}	152.9	100.6	126.8	36.949	59.830

Water Flow Rates							
Time	0	60	120	180	325	AVG	STDEV
R-5	18.0	18.5	18.0	17.5	17.5	18.0	0.418
R-6	18.0	16.5	17.0	17	17	16.8	0.548

NaCl Flow Rates				
Time	0	330	AVG	STDEV
R-5	0.18	0.20	0.19	0.01
R-6	0.20	0.20	0.20	0.00

Appendix G.1-2
NaCl Tracer Study Results
Conductivity Measurements

0.5-L Reactors

Time (min)	Conductivity (ms) R-5	Time (min)	Conductivity (ms) R-6
0	1.382	0	1.456
15	1.941	10	1.762
30	2.31	25	2.08
60	2.71	40	2.29
90	2.88	70	2.5
160	3.04	100	2.59
285	3.05	160	2.65

System Tracer Study
500-mL Reactors

Reactor 5

Time	[A]out	[A]out	Area Curve 1	[Aout]/[Ain]	Area Curve 2	Time Curve 3,4	ΔAout/Δt Curve 3	Area Curve 3	Mean top	Mean bottom	Variance top	Variance bottom	Δ(Aout/Ain)/Δt Curve 4	Area Curve 4	Mean top	Mean bottom	Variance top	Variance bottom
0	1.382	0	0.00	0.000	0.000	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
15	1.941	0.559	20.82	0.335	12.486	7.5	0.037	0.280	4.193	0.559	31.444	0.559	0.022	0.168	2.514	0.335	18.856	0.335
30	2.31	0.928	13.86	0.556	8.312	22.5	0.025	0.464	8.303	0.369	186.806	0.369	0.015	0.278	4.979	0.221	112.021	0.221
60	2.71	1.328	16.19	0.796	9.707	45	0.013	0.569	18.000	0.400	810.000	0.400	0.008	0.341	10.794	0.240	485.729	0.240
90	2.88	1.498	7.64	0.898	4.580	75	0.006	0.285	12.750	0.170	956.250	0.170	0.003	0.171	7.646	0.102	573.430	0.102
160	3.04	1.658	6.27	0.994	3.761	125	0.002	0.278	20.000	0.160	2500.000	0.160	0.001	0.167	11.993	0.096	1499.164	0.096
285	3.05	1.668	0.57	1.000	0.344	222.5	0.000	0.148	2.225	0.010	495.062	0.010	0.000	0.089	1.334	0.006	296.872	0.006
			65.4		39.2			2.024	65.47	1.668	4979.56	1.668		1.214	39.26	1.000	2986.07	1.000
			39.4						mean = 39.25		variance = 1445				mean = 39.25		variance = 1445	
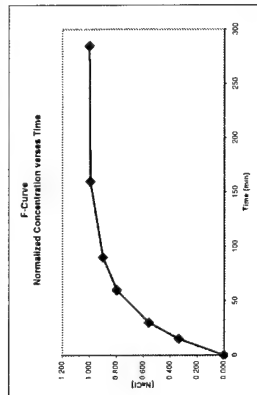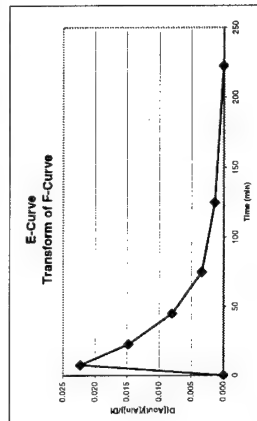
Reactor 5:

	Curve 1	F-Curve	Curve 3	E-Curve
Area	65.35	39.19	2.02	1.21
Mean	39.39	39.19	39.25	39.25
Variance	--	--	1444.7	1444.7

From Graph:

t(x%)	t (min)
t10	4.5
t50	37.7
t90	50.1
t95	152.9

F-Curve — Normalized Concentration verses Time

E-Curve — Transform of F-Curve

Reactor 6

Time	[A]out	[A]out	Area Curve 1	[Aout]/[Ain]	Area Curve 2	Time Curve 3,4	ΔAout/Δt Curve 3	Area Curve 3	Mean top	Mean bottom	Variance top	Variance bottom	ΔAout/Ain/Δt Curve 4	Area Curve 4	Mean top	Mean bottom	Variance top	Variance bottom
0	1.456	0	0.00	0.000	0.000	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
10	1.762	0.306	10.48	0.255	8.726	5	0.031	0.153	1.530	0.306	7.650	0.306	0.025	0.127	1.274	0.255	6.369	0.255
25	2.08	0.624	11.04	0.519	9.193	17.5	0.021	0.389	5.565	0.318	97.388	0.318	0.018	0.323	4.633	0.265	81.078	0.265
40	2.29	0.834	7.08	0.694	5.896	32.5	0.014	0.264	6.825	0.210	221.813	0.210	0.012	0.220	5.682	0.175	184.666	0.175
70	2.5	1.044	7.86	0.869	6.548	55	0.007	0.315	11.550	0.210	635.250	0.210	0.006	0.262	9.616	0.175	528.865	0.175
100	2.59	1.134	3.36	0.944	2.801	85	0.003	0.150	7.650	0.090	650.250	0.090	0.002	0.125	6.369	0.075	541.353	0.075
160	2.65	1.194	2.23	0.994	1.856	130	0.001	0.120	7.800	0.060	1014.000	0.060	0.001	0.100	6.494	0.050	844.186	0.050
			42.1		35.02			1.391	40.92	1.194	2626.4	1.194		1.158	34.07	0.994	2186.52	0.994
			23.10						mean = 34.27		variance = 1025				mean = 34.27		variance = 1025	

Reactor 6:

	Curve 1	F-Curve	Curve 3	E-Curve
Area	42.07	35.02	1.39	1.16
Mean	23.10	35.02	34.27	34.27
Variance	--	--	1025.1	1025.1

From Graph:

t(x%)	t (min)
t10	3.9
t50	24.1
t90	95.3
t95	100.6

F-Curve — Normalized Concentration verses Time

E-Curve — Transform of F-Curve

$C_o =$	3.050	2.972	2.935
Norm $C_o =$	1.668	1.590	1.554
Tau=	37.381	29.889	26.946
Z=	1	2	3

Reactor 5

Time (min)	Conductivity (ms)	N=1	(obs-model)²	N=2	(obs-model)²	N=3	(obs-model)²	N=4	(obs-model)²
0	0	0.000	0.0E+00	0.000	0.0E+00	0.000	0.0E+00	0.000	0.0E+00
15	0.559	0.551	6.1E-05	0.422	1.9E-02	0.365	3.6E-02	0.158	1.6E-01
30	0.928	0.920	6.1E-05	0.948	3.6E-04	1.008	6.4E-03	0.745	3.4E-02
60	1.328	1.333	2.1E-05	1.446	1.4E-02	1.495	2.8E-02	1.520	3.7E-02
90	1.496	1.517	3.8E-04	1.563	4.2E-03	1.550	2.7E-03	1.657	2.5E-02
160	1.658	1.645	1.8E-04	1.590	4.7E-03	1.554	1.1E-02	1.672	2.1E-04
285	1.668	1.667	1.5E-06	1.590	6.1E-03	1.554	1.3E-02	1.672	1.5E-05
		SUM	0.001		0.048		0.099		0.257

3.054	3.226	3.207	3.503	3.503
1.672	1.844	1.825	2.121	2.121
35.115	64.872	53.261	32.066	35.231
4	5	6	9	10

N=4	(obs-model)²	N=5	(obs-model)²	N=6	(obs-model)²	N=9	(obs-model)²	N=10	(obs-model)²
0.000	0.0E+00	0.000	0.0E+00	0.000	0.0E+00	0.000	0.0E+00	0.000	0.0E+00
0.158	1.6E-01	0.012	3.0E-01	0.006	3.1E-01	0.060	2.5E-01	0.060	2.5E-01
0.745	3.4E-02	0.157	5.9E-01	0.126	6.4E-01	0.988	3.6E-03	0.989	3.7E-03
1.520	3.7E-02	0.906	1.8E-01	0.919	1.7E-01	2.092	5.8E-01	2.092	5.8E-01
1.657	2.5E-02	1.514	2.7E-04	1.556	3.4E-03	2.121	3.9E-01	2.121	3.9E-01
1.672	2.1E-04	1.833	3.1E-02	1.820	1.8E-02	2.121	2.1E-01	2.121	2.1E-01
1.672	1.5E-05	1.844	1.3E-02	1.825	3.1E-02	2.121	2.1E-01	2.121	2.1E-01
	0.257		1.133		1.170		1.631		1.645

Series Model
Reactor 3

$C_o =$	2.657	2.605	2.587
Norm $C_o =$	1.201	1.149	1.131
Z=	34.054	28.070	26.670
	1	2	3

Reactor 6

Time (min)	Conductivity (ms)	N=1	(obs-model)²	N=2	(obs-model)²	N=3	(obs-model)²	N=4	(obs-model)²
0	0.306	0.000	0.0E+00	0.000	0.0E+00	0.000	0.0E+00	0.000	0.0E+00
10	0.624	0.306	1.2E-07	0.184	1.5E-02	0.118	3.5E-02	0.077	5.3E-02
25	0.834	0.625	4.7E-07	0.611	1.6E-04	0.603	4.3E-04	0.592	1.9E-03
40	1.044	0.830	1.5E-05	0.893	3.5E-03	0.934	1.0E-02	0.961	1.6E-02
70	1.134	1.047	1.1E-05	1.102	3.3E-03	1.114	4.8E-03	1.115	5.0E-03
100	1.194	1.137	1.2E-05	1.141	4.9E-05	1.130	1.9E-05	1.122	1.5E-04
160		1.190	1.4E-05	1.148	2.1E-03	1.131	4.0E-03	1.122	5.2E-03
		SUM	0.000		0.024		0.055		0.080

2.578	2.572	2.568	2.560	2.556
1.122	1.116	1.112	1.104	1.100
26.271	26.146	26.054	25.763	26.105
4	5	6	8	10

N=4	(obs-model)²	N=5	(obs-model)²	N=6	(obs-model)²	N=8	(obs-model)²	N=10	(obs-model)²
0.000	0.0E+00	0.000	0.0E+00	0.000	0.0E+00	0.000	0.0E+00	0.000	8.7E-02
0.077	5.3E-02	0.050	6.5E-02	0.033	7.4E-02	0.016	8.4E-02	0.011	3.0E-02
0.592	1.9E-03	0.581	1.9E-03	0.572	2.7E-03	0.567	3.3E-03	0.569	4.0E-02
0.961	1.6E-02	0.981	2.1E-02	0.997	2.6E-02	1.023	3.6E-02	1.034	3.1E-03
1.115	5.0E-03	1.113	4.8E-03	1.110	4.4E-03	1.103	3.5E-04	1.100	1.2E-03
1.122	1.5E-04	1.116	3.2E-04	1.112	5.0E-04	1.104	9.3E-04	1.100	8.9E-03
1.122	5.2E-03	1.116	6.1E-03	1.112	6.8E-03	1.104	8.2E-03	1.100	0.143
0.080		0.100		0.115		0.127		0.136	

Series Model
Reactor 6

Series Model
Reactor 6

Parameter	Units	R-1	R-2
Volume	L	0.58	0.58
Flow Rate	mL/min	18.0	19.0
$\tau_{nominal}$	min	32.2	30.5
Mean t (F-Curve)	min	40.4	41.0
Mean t (E-Curve)	min	39.3	39.3
Variance	min^2	1337	1291
t_{10}	min	4.3	5.1
t_{50}	min	29.3	28.1
t_{90}	min	88.2	100.4
t_{95}	min	148.0	120.5
$3*\tau_{nominal}$	min	96.7	91.6
Z		1	1
C_0		1.10	0.94
τ_{model}	min	41.0	41.0
$\tau_{model}/\tau_{nominal}$	min/min	1.27	1.34
$(Variance_\tau)^2$	min^4	28.86	23.25
$Variance_\tau$	min^2	5.4	4.8
+Var Ratio		1.09	1.16
-Var Ratio		1.53	1.60
t_{90}/t_{10}	min/min	20.5	19.7
mean $t/\tau_{nominal}$	min/min	1.25	1.34
$t_{10}/\tau_{nominal}$	min/min	0.133	0.167

	R1	R2	AVG	STDEV	95% CI
$\tau_{nominal}$	32.2	30.5	31.4	1.199	1.942
Mean t (E-Curve)	39.3	39.3	39.3	0.013	0.021
t_{10}	4.3	5.1	4.7	0.569	0.921
t_{50}	29.3	28.1	28.7	0.905	1.465
t_{90}	88.2	100.4	94.3	8.589	13.908
t_{95}	148.0	120.5	134.3	19.438	31.476

Water Flow Rates				
Time	0	60	AVG	STDEV
R-1	19.0	17.0	18.0	1.414
R-2	19.0	19.0	19.0	0.000
NaCl Flow Rates				
Time	0	370	AVG	STDEV
R-1	0.20	0.20	0.20	0.00
R-2	0.20	0.20	0.20	0.00

0.5-L Reactors

Time	Absorbance	
(min)	R-1	R-2
0	0.028	0.007
10	0.098	0.190
15	0.215	0.280
20	0.320	0.354
25	0.400	0.442
30	0.483	0.506
40	0.612	0.588
50	0.693	0.647
60	0.840	0.758
100	1.021	0.844
120	1.033	0.890
140	1.042	0.931
160	1.066	0.903
180	1.095	0.929

System Tracer Study
500-mL Reactors

Appendix G.2-3
PNP Tracer Study
Residence Time Distribution

Reactor 1

Time	[A]out	[A]out Norm	Area Curve 1	[Aout]/[Ain]	Area Curve 2	Time Curve 3,4	Δ[Aout]/Δt Curve 3	Area Curve 3	Δ(Aout/Ain)/Δt Curve 4	Area Curve 4 (F)	Mean top (F)	Mean bottom (F)	Variance top (F)	Variance bottom (F)	Area Curve 4 (E)	Mean top (E)	Mean bottom (E)	Variance top (E)	Variance bottom (E)
0	0.098	0.000	0.000	0.000	0.000	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
5	0.215	0.117	4.74	0.116	4.709	2.5	0.023	0.059	0.023	0.058	0.293	0.117	0.731	0.117	0.058	0.291	0.116	0.727	0.116
10	0.320	0.222	4.18	0.221	4.157	7.5	0.021	0.111	0.021	0.110	0.788	0.105	5.906	0.105	0.110	0.783	0.104	5.874	0.104
15	0.400	0.302	3.72	0.300	3.697	12.5	0.016	0.093	0.016	0.092	1.000	0.080	12.500	0.080	0.092	0.994	0.080	12.431	0.080
20	0.483	0.385	3.31	0.383	3.292	17.5	0.017	0.082	0.017	0.081	1.453	0.083	25.419	0.083	0.081	1.444	0.083	25.279	0.083
30	0.612	0.514	5.56	0.511	5.530	25	0.013	0.148	0.013	0.147	3.225	0.129	80.625	0.129	0.147	3.207	0.128	80.181	0.128
40	0.693	0.595	4.51	0.592	4.486	35	0.008	0.105	0.008	0.104	2.835	0.081	99.225	0.081	0.104	2.819	0.081	98.678	0.081
50	0.840	0.742	3.37	0.738	3.352	45	0.015	0.114	0.015	0.113	6.615	0.147	297.675	0.147	0.113	6.579	0.146	296.034	0.146
90	1.021	0.923	6.92	0.918	6.884	70	0.005	0.385	0.005	0.382	12.670	0.181	886.900	0.181	0.382	12.600	0.180	882.010	0.180
110	1.033	0.935	1.53	0.930	1.522	100	0.001	0.051	0.001	0.051	1.200	0.012	120.000	0.012	0.051	1.193	0.012	119.338	0.012
130	1.042	0.944	1.32	0.939	1.314	120	0.000	0.011	0.000	0.010	1.080	0.009	129.600	0.009	0.010	1.074	0.009	128.886	0.009
150	1.066	0.968	0.99	0.963	0.985	140	0.001	0.017	0.001	0.016	3.360	0.024	470.400	0.024	0.016	3.341	0.024	467.807	0.024
170	1.095	0.997	0.46	0.992	0.458	160	0.001	0.027	0.001	0.026	4.640	0.029	742.400	0.029	0.026	4.614	0.029	738.307	0.029
			40.6		40.4			1.199		1.193	39.16	0.997	2671.4	0.997	1.193	38.94	0.992	2655.6	0.992
			21.5								mean = 39.16	mean = 39.3	variance = 2671.4	variance = 1337.5		mean = 38.94	mean = 39.3	variance = 2655.6	variance = 1337

Reactor 1:

	Curve 1	F-Curve	Curve 3	E-Curve
Area	40.61	40.39	1.20	1.19
Mean	21.52	40.39	39.28	39.28
Variance	--	--	1337.5	1337.5

From Graph:

t(x%)	t (min)
110	4.3
150	29.3
190	88.2
195	148.0

F-Curve: Normalized Concentration verses Time

E-Curve: Transform of F-Curve

Reactor 2

Time	[A]out	[A]out Norm	Area Curve 1	[Aout]/[Ain]	Area Curve 2	Time Curve 3,4	Δ[Aout]/Δt Curve 3	Area Curve 3	Δ(Aout/Ain)/Δt Curve 4	Area Curve 4 (F)	Mean top (F)	Mean bottom (F)	Variance top (F)	Variance bottom (F)	Area Curve 4 (E)	Mean top (E)	Mean bottom (E)	Variance top (E)	Variance bottom (E)
0	0.007	0	0.000	0.000	0.000	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
10	0.19	0.183	8.42	0.196	9.020	5	0.018	0.092	0.020	0.098	0.915	0.183	4.575	0.183	0.098	0.980	0.196	4.900	0.196
15	0.28	0.273	3.53	0.292	3.779	12.5	0.018	0.091	0.019	0.097	1.125	0.090	14.063	0.090	0.097	1.205	0.096	15.062	0.096
20	0.354	0.347	3.12	0.372	3.340	17.5	0.015	0.082	0.016	0.088	1.295	0.074	22.663	0.074	0.088	1.387	0.079	24.273	0.079
25	0.442	0.435	2.71	0.466	2.906	22.5	0.018	0.081	0.019	0.087	1.980	0.088	44.550	0.088	0.087	2.121	0.094	47.717	0.094
30	0.506	0.499	2.33	0.534	2.499	27.5	0.013	0.076	0.014	0.081	1.760	0.064	48.400	0.064	0.081	1.885	0.069	51.841	0.069
40	0.588	0.581	3.94	0.622	4.216	35	0.008	0.105	0.009	0.112	2.870	0.082	100.450	0.082	0.112	3.074	0.088	107.591	0.088
50	0.647	0.64	3.23	0.685	3.461	45	0.006	0.071	0.006	0.076	2.655	0.059	119.475	0.059	0.076	2.844	0.063	127.968	0.063
60	0.758	0.751	2.38	0.804	2.551	55	0.011	0.085	0.012	0.091	6.105	0.111	335.775	0.111	0.091	6.539	0.119	359.644	0.119
100	0.844	0.837	5.59	0.896	5.982	80	0.002	0.265	0.002	0.284	6.880	0.086	550.400	0.086	0.284	7.369	0.092	589.526	0.092
120	0.89	0.883	1.47	0.946	1.577	110	0.002	0.045	0.002	0.048	5.060	0.046	556.600	0.046	0.048	5.420	0.049	596.167	0.049
140	0.931	0.924	0.60	0.990	0.645	130	0.002	0.044	0.002	0.047	5.330	0.041	692.900	0.041	0.047	5.709	0.044	742.156	0.044
160	0.903	0.896	0.47	0.960	0.506	150	-0.001	0.007	-0.001	0.007	-4.200	-0.028	-630.000	-0.028	0.007	-4.499	-0.030	-674.795	-0.030
180	0.929	0.922	0.49	0.988	0.528	170	0.001	-0.001	0.001	-0.001	4.420	0.026	751.400	0.026	-0.001	4.734	0.028	804.814	0.028
			38.3		41.01			1.040		1.114	36.20	0.922	2611.3	0.922	1.114	38.77	0.988	2796.87	0.988
			21.03								mean = 36.20	mean = 39.26	variance = 2611.3	variance = 1291		mean = 38.77	mean = 39.26	variance = 2796.87	variance = 1291

Reactor 2:

	Curve 1	F-Curve	Curve 3	E-Curve
Area	38.29	41.01	1.04	1.11
Mean	21.03	41.01	39.26	39.26
Variance	--	--	1291.0	1291.0

From Graph:

t(x%)	t (min)
110	5.1
150	28.1
190	100.4
195	120.5

F-Curve: Normalized Concentration verses Time

E-Curve: Transform of F-Curve

Reactor 1

	N=1	N=2	N=3	N=4	N=5	N=6	N=7	N=8	N=9	N=10
C_0 =	1.104	1.047	1.030	1.019	1.009	1.001	0.993	0.986	0.979	0.978
Norm C_0 =	1.006	0.949	0.932	0.921	0.911	0.903	0.895	0.888	0.881	0.880
Tau =	41.005	31.788	29.762	28.812	28.165	27.612	27.071	26.497	25.842	29.393
Z =	1	2	3	4	5	6	7	8	9	10

Time (min)	Absorbance	N=1	(obs-model)²	N=2	(obs-model)²	N=3	(obs-model)²	N=4	(obs-model)²	N=5	(obs-model)²	N=6	(obs-model)²	N=7	(obs-model)²	N=8	(obs-model)²	N=9	(obs-model)²	N=10	(obs-model)²
0	0	0.000	0.0E+00	0.000	0.0E+00	0.000	0.0E+00	0.000	0.0E+00	0.000	0.0E+00	0.000	0.0E+00	0.000	0.0E+00	0.000	0.0E+00	0.000	0.0E+00	0.000	0.0E+00
5	0.117	0.115	2.5E-06	0.008	4.8E-03	0.014	1.6E-02	0.005	1.5E-02	0.002	1.3E-02	0.001	1.3E-02	0.000	1.4E-02	0.000	1.4E-02	0.000	1.4E-02	0.000	1.4E-02
10	0.222	0.218	1.9E-05	0.125	9.4E-03	0.076	2.1E-02	0.048	2.1E-02	0.032	3.0E-02	0.021	4.0E-02	0.015	4.3E-02	0.011	4.5E-02	0.008	4.6E-02	0.008	4.6E-02
15	0.302	0.308	3.7E-05	0.231	5.0E-03	0.181	1.5E-02	0.146	2.4E-02	0.120	3.3E-02	0.101	4.0E-02	0.088	4.6E-02	0.079	5.0E-02	0.074	5.0E-02	0.074	5.0E-02
20	0.385	0.388	9.8E-06	0.340	2.0E-03	0.305	6.3E-03	0.279	1.1E-02	0.259	1.6E-02	0.245	2.0E-02	0.236	2.2E-02	0.232	2.3E-02	0.235	2.3E-02	0.235	2.3E-02
30	0.514	0.522	6.0E-05	0.534	4.0E-04	0.543	8.2E-04	0.551	1.3E-03	0.560	2.1E-03	0.572	3.4E-03	0.587	5.4E-03	0.606	8.5E-03	0.630	1.3E-02	0.630	1.3E-02
40	0.595	0.625	9.9E-04	0.680	7.2E-03	0.715	1.4E-02	0.741	2.1E-02	0.762	2.8E-02	0.781	3.4E-02	0.797	4.1E-02	0.811	4.7E-02	0.824	5.2E-02	0.824	5.2E-02
50	0.742	0.708	1.1E-03	0.780	1.4E-03	0.819	6.9E-03	0.843	1.0E-02	0.857	1.3E-02	0.866	1.6E-02	0.871	1.7E-02	0.873	1.7E-02	0.872	1.7E-02	0.872	1.7E-02
90	0.923	0.894	8.7E-04	0.927	1.8E-05	0.919	1.3E-05	0.919	1.3E-05	0.911	1.3E-05	0.903	4.0E-04	0.895	7.6E-04	0.888	1.2E-03	0.881	1.8E-03	0.880	1.8E-03
110	0.935	0.937	3.2E-06	0.942	4.6E-05	0.931	1.6E-05	0.921	2.1E-04	0.911	5.5E-04	0.903	1.0E-03	0.895	1.6E-03	0.888	2.2E-03	0.881	3.0E-03	0.880	3.0E-03
130	0.944	0.963	3.7E-05	0.947	7.8E-06	0.932	1.4E-04	0.921	5.4E-04	0.911	1.1E-03	0.903	1.7E-03	0.895	2.4E-03	0.888	3.1E-03	0.881	4.0E-03	0.880	4.0E-03
150	0.968	0.960	1.4E-04	0.948	3.8E-04	0.932	1.3E-03	0.921	2.2E-03	0.911	3.2E-03	0.903	4.2E-03	0.895	5.3E-03	0.888	6.4E-03	0.881	7.7E-03	0.880	7.7E-03
170	0.997	0.990	5.4E-06	0.949	2.3E-03	0.932	4.2E-03	0.921	5.8E-03	0.911	7.3E-03	0.903	8.8E-03	0.895	1.0E-02	0.888	1.2E-02	0.881	1.4E-02	0.880	1.4E-02
SUM			0.004		0.034		0.080		0.120		0.154		0.183		0.208		0.229		0.247		0.247
r^2			0.998		0.985		0.966		0.950		0.936		0.924		0.913		0.902		0.892		0.892

Series Model
Reactor 1

Series Model
Reactor 1

Reactor 2

	N=1	N=2	N=3	N=4	N=5	N=6	N=7	N=8	N=9	N=10
C_0 =	0.941	0.889	0.867	0.851	0.839	0.830	0.823	0.816	0.814	0.814
Norm C_0 =	0.934	0.882	0.860	0.844	0.832	0.823	0.816	0.811	0.807	0.807
Tau =	41.036	32.135	29.503	27.948	26.875	26.116	25.576	25.187	24.900	27.360
Z =	1	2	3	4	5	6	7	8	9	10

Time (min)	Absorbance	N=1	(obs-model)²	N=2	(obs-model)²	N=3	(obs-model)²	N=4	(obs-model)²	N=5	(obs-model)²	N=6	(obs-model)²	N=7	(obs-model)²	N=8	(obs-model)²	N=9	(obs-model)²	N=10	(obs-model)²
0	0	0.000	0.0E+00	0.000	0.0E+00	0.000	0.0E+00	0.000	0.0E+00	0.000	0.0E+00	0.000	0.0E+00	0.000	0.0E+00	0.000	0.0E+00	0.000	0.0E+00	0.000	0.0E+00
10	0.183	0.202	3.6E-04	0.114	4.8E-03	0.072	1.2E-02	0.048	1.8E-02	0.034	2.2E-02	0.025	2.5E-02	0.018	2.7E-02	0.013	2.9E-02	0.010	3.0E-02	0.010	3.0E-02
15	0.273	0.266	1.7E-04	0.211	3.8E-03	0.170	1.1E-02	0.144	1.7E-02	0.126	2.2E-02	0.111	2.6E-02	0.100	3.0E-02	0.089	3.4E-02	0.080	3.7E-02	0.080	3.7E-02
20	0.347	0.360	1.7E-04	0.312	1.2E-03	0.286	3.7E-03	0.272	5.6E-03	0.264	6.9E-03	0.258	7.9E-03	0.253	8.8E-03	0.248	9.8E-03	0.243	1.1E-02	0.243	1.1E-02
25	0.435	0.426	8.2E-05	0.406	8.2E-04	0.402	1.1E-03	0.405	8.8E-04	0.413	4.8E-04	0.422	1.8E-04	0.430	2.6E-05	0.437	4.0E-06	0.443	6.6E-05	0.443	6.6E-05
30	0.499	0.484	2.2E-04	0.491	6.3E-05	0.506	4.7E-05	0.525	6.7E-04	0.545	2.1E-03	0.564	4.3E-03	0.581	6.7E-03	0.596	9.3E-03	0.608	1.2E-02	0.608	1.2E-02
40	0.581	0.581	1.5E-07	0.627	2.1E-03	0.664	6.8E-03	0.695	1.3E-02	0.719	1.9E-02	0.737	2.4E-02	0.750	2.9E-02	0.760	3.2E-02	0.767	3.5E-02	0.767	3.5E-02
50	0.64	0.658	3.1E-04	0.720	6.5E-03	0.769	1.6E-02	0.782	2.0E-02	0.794	2.4E-02	0.800	2.6E-02	0.802	2.6E-02	0.802	2.6E-02	0.802	2.6E-02	0.802	2.6E-02
60	0.751	0.717	1.1E-03	0.782	9.7E-04	0.811	3.5E-03	0.820	4.5E-03	0.821	5.0E-05	0.818	2.2E-05	0.814	1.9E-04	0.810	3.5E-03	0.806	2.6E-02	0.806	2.6E-02
100	0.837	0.852	2.2E-04	0.869	1.0E-03	0.858	4.5E-04	0.844	5.4E-04	0.832	2.5E-05	0.823	2.0E-04	0.816	4.2E-04	0.811	6.7E-04	0.807	9.0E-04	0.807	9.0E-04
120	0.883	0.883	2.4E-07	0.878	1.9E-05	0.860	5.4E-04	0.844	1.5E-03	0.832	2.6E-03	0.823	3.6E-03	0.816	4.4E-03	0.811	5.2E-03	0.807	5.8E-03	0.807	5.8E-03
140	0.924	0.903	4.5E-04	0.880	1.9E-03	0.860	4.1E-03	0.844	6.3E-03	0.832	8.4E-03	0.823	1.0E-02	0.816	1.2E-02	0.811	1.4E-02	0.807	1.4E-02	0.807	1.4E-02
160	0.896	0.915	3.5E-04	0.881	2.1E-04	0.860	1.3E-03	0.844	2.7E-03	0.832	4.1E-03	0.823	5.3E-03	0.816	6.3E-03	0.811	7.2E-03	0.807	7.9E-03	0.807	7.9E-03
180	0.922	0.922	1.3E-10	0.882	1.6E-03	0.860	3.8E-03	0.844	6.0E-03	0.832	8.0E-03	0.823	9.7E-03	0.816	1.1E-02	0.811	1.3E-02	0.807	1.3E-02	0.807	1.3E-02
SUM			0.063		0.025		0.063		0.096		0.124		0.147		0.165		0.182		0.195		0.196
r^2			0.997		0.983		0.958		0.935		0.917		0.902		0.890		0.879		0.871		0.871

Series Model
Reactor 7

Series Model
Reactor 7

Appendix G
Morril Dispersion Index

The Morril Disperion Index (MDI) is defined in Equation G.1. The following derivation is the theoretical calculation of this index from the CMFTR model.

$$MDI = \frac{t_{90}}{t_{10}} \qquad (G.1)$$

The MDI for a CMFTR can be derived as follows:

$$\frac{C}{C_0} = 1 - \exp(\frac{-t}{\tau})$$

For t_{10}:

$$\frac{C}{C_0} = 0.10 = 1 - \exp(\frac{-t_{10}}{\tau})$$

$$0.9 = \exp(\frac{-t_{10}}{\tau})$$

$$\frac{t_{10}}{\tau} = 0.105$$

For t_{90}:

$$\frac{C}{C_0} = 0.90 = 1 - \exp(\frac{-t_{90}}{\tau})$$

$$0.1 = \exp(\frac{-t_{90}}{\tau})$$

$$\frac{t_{90}}{\tau} = 2.303$$

Substituting and solving for the MDI:

$$MDI = \frac{t_{90}}{t_{10}}$$

$$MDI = \frac{\dfrac{t_{90}}{\tau}}{\dfrac{t_{10}}{\tau}} = \frac{2.303}{0.105}$$

$$MDI = 21.9$$

Appendix H
Water Quality Analysis

Raw Water

	Date	pH	Turbidity (NTU)	Total Hardness (mg/L as CaCO$_3$)	Ca^{2+} Hardness (mg/L as CaCO$_3$)	Mg^{2+} Hardness (mg/L as CaCO$_3$)	Alkalinity (mg/L as CaCO$_3$)	TOC (mg/L)	DOC (mg/L)	UV$_{254}$ (cm^{-1})	SUVA
3	7-May-00	7.89	3.35	430	200	230	228	5.31	5.33	0.059	0.011
6	1-Aug-00	8.71	3.06	290	120	170	224	ND	ND	NA	NA
7	12-Sep-00	8.38	3.01	330	140	190	110	4.12	4.18	NA	NA
9	26-Sep-00	7.68	3.26	NA	NA	NA	257	NA	NA	NA	NA
11	24-Feb-01	7.56	2.66	400	180	220	258	4.84	5.22	0.078	0.015
	Maximum	8.71	3.35	430	200	230	258	5.31	5.33	0.059	0.011
	Minimum	7.56	2.66	290	120	170	110	4.12	4.18	0.078	0.015
	Median	7.89	3.06	365	160	205	228	4.84	5.22	0.069	0.013
	Average	8.04	3.07	363	160	203	215	4.76	4.91	0.069	0.013

Filtered Water

Experiment	Date	pH	Turbidity (NTU)	Total Hardness (mg/L as CaCO$_3$)	Ca^{2+} Hardness (mg/L as CaCO$_3$)	Mg^{2+} Hardness (mg/L as CaCO$_3$)	Alkalinity (mg/L as CaCO$_3$)	TOC (mg/L)	DOC (mg/L)	UV$_{254}$ (cm^{-1})	SUVA
1	3-Apr-00	6.26	0.26	NA	NA	NA	NA	NA	NA	NA	NA
2	22-Apr-00	6.22	0.23	430	210	220	20	ND	ND	0.034	ND
4	26-Jun-00	5.96	0.33	NA	NA	NA	NA	2.99	2.94	0.068	0.023
5	26-Jul-00	6.56	0.21	270	120	150	96	2.35	3.59	0.013	0.004
8	20-Sep-00	6.41	0.39	410	200	210	110	3.07	3.04	0.054	0.018
12	3-Mar-01	6.22	0.14	420	180	240	66	3.02	2.92	0.037	0.013
13	7-Apr-01	6.10	0.13	390	180	210	173	NA	NA	NA	NA
	Maximum	6.56	0.39	430	210	240	173	3.07	3.59	0.013	0.013
	Minimum	5.96	0.13	270	120	150	20	2.35	2.92	0.068	0.004
	Median	6.22	0.23	410	180	210	96	3.01	2.99	0.037	0.015
	Average	6.25	0.24	384	178	206	93	2.86	3.12	0.041	0.014

Note: NA = Not Analyzed.
SUVA = UV$_{254}$/DOC. This is a measure of the molecular weight distribution of NOM in the water (AWWA, 1999).

Appendix I.1
Disinfectant Decay Analysis
Raw Water

Raw Waters

	Reactor Data			Flow Data		NaOCl Data							1st Order Modeling Info						2nd Order Modeling Info			
Date	Volume (mL)	t_{mean} (min)	Q_s (mL/min)	Q_4 (mL/min)	Q_2 (mL/min)	C_3 (mg/L)	C_4 (mg/L)	C_1 (mg/L)	$C_{consumed}$ (mg/L)	C_{calc}	$(C_{calc}-C_1)^2$	C_1Q_1/C_4Q_4	k	SSE	r^2	C_{calc}	$(C_{calc}-C_1)^2$	k	SSE	r^2
7-May-00 crypto	2250	81.3	27.4	27.7	0.29	0	790	5.65	2.62	5.56	0.01	0.683	0.00600743	8.7	0.66	5.55	0.01	0.0011	8.77	0.69
	2250	87.7	25.4	25.7	0.25	0	790	7.22	0.48	5.04	4.74	0.938				5.16	4.24			
	2250	90.0	24.7	25.0	0.29	0	615	5.02	2.12	4.63	0.15	0.703				4.84	0.03			
	1230	48.6	25.1	25.3	0.23	0	415	2.50	1.27	2.92	0.17	0.663				3.22	0.52			
	1230	60.4	20.1	20.4	0.26	0	790	6.79	3.30	7.40	0.37	0.673				6.93	0.02			
	1230	65.3	18.6	18.8	0.24	0	615	4.06	3.77	5.63	2.46	0.518				5.61	0.02			
	1230	57.8	21.1	21.3	0.19	0	415	1.78	1.83	2.68	0.81	0.493				3.03	1.56			
1-Aug-00 crypto	2250	83.2	26.7	27.1	0.35	0	780	4.15	5.94	2.66	2.21	0.411	0.03352349	6.2	0.27	2.73	2.39	0.0119	4.49	0.29
	2250	89.0	24.9	25.3	0.38	0	550	3.08	5.19	2.08	1.01	0.373				2.37	0.51			
	2250	89.8	24.8	25.1	0.25	0	550	1.39	4.10	1.37	0.00	0.253				1.85	0.21			
	1230	56.0	21.7	22.0	0.25	0	780	3.28	5.60	3.09	0.04	0.369				2.98	0.09			
	1230	59.7	20.3	20.6	0.29	0	550.0	2.83	4.92	2.58	0.06	0.365				2.68	0.02			
	1230	67.1	18.0	18.3	0.33	0	360.0	1.90	4.58	1.99	0.01	0.293				2.29	0.16			
	580	26.8	21.4	21.6	0.23	0	780	3.51	4.78	4.37	0.74	0.423				3.77	0.07			
	580	29.0	19.7	20.0	0.27	0	550	2.56	4.88	3.77	1.46	0.344				3.42	0.73			
	580	32.1	17.8	18.1	0.29	0	360	1.97	3.80	2.78	0.66	0.341				2.80	0.68			
26-Sep-00 crypto	2250	87.7	25.3	25.7	0.37	0	74200	1080.00	-10.50	1066.69	177.09	1.010	0.00003	8691.6	0.99	1068.50	132.32	0.00000001	21605.19	0.86
	2250	89.1	24.8	25.3	0.46	0	31500	537.33	36.30	572.11	1209.33	0.937				573.34	1296.82			
	2250	87.0	25.5	25.9	0.35	0	10680	141.47	3.13	144.23	7.60	0.978				144.59	9.71			
	1230	56.4	21.5	21.8	0.30	0	74200	1104.67	-83.57	1019.38	7275.16	1.082				1020.51	7082.35			
	1230	53.8	22.5	22.9	0.36	0	31500	500.00	-3.94	495.26	22.43	0.965				495.93	16.56			
	1230	57.6	21.1	21.4	0.27	0	10680	130.17	4.77	134.70	20.56	0.965				134.93	22.62			
	580	28.2	20.4	20.6	0.19	0	74200	578.65	106.05	684.12	1124.59	0.845				684.57	11218.90			
	580	28.4	20.1	20.4	0.33	0	31500	498.25	10.56	508.38	102.57	0.979				508.74	109.98			
	580	29.6	19.3	19.6	0.31	0	10680	127.40	41.43	168.68	1704.25	0.755				168.82	1715.93			
24-Feb-01 crypto	2250	158.1	14.1	14.2	0.13	0	5539	37.93	12.67	40.04	4.44	0.750	0.00166912	640.3	0.91	44.67	45.36	0.000019	688.42	0.91
	2250	165.1	13.5	13.6	0.13	0	8164	65.20	12.67	61.05	17.25	0.837				64.81	0.15			
	2250	160.5	13.9	14.0	0.12	0	12984	89.47	21.66	87.65	3.30	0.805				87.83	2.69			
	1230	111.6	10.9	11.0	0.12	0	5539	44.10	16.22	50.84	45.48	0.731				54.15	101.10			
	1230	93.8	13.0	13.1	0.11	0	8164	71.00	-2.50	59.23	138.63	1.036				61.76	85.31			
	1230	121.7	10.0	10.1	0.11	0	12984	124.00	17.27	117.42	43.23	0.878				112.36	135.48			
	580	63.0	9.1	9.2	0.10	0	5539	48.25	11.95	54.47	38.74	0.801				56.43	66.88			
	580	47.9	12.0	12.1	0.10	0	8164	57.13	10.34	62.47	28.55	0.847				63.60	44.47			
	580	54.7	10.5	10.6	0.11	0	12994	105.45	29.16	123.36	320.67	0.783				119.84	206.98			

Low Detention Time Run

	Reactor Data			Flow Data		NaOCl Data							1st Order Modeling Info						2nd Order Modeling Info			
24-Mar-01	145	3.7	39.1	39.6	0.45	0	341	2.93	0.95	3.32	0.15	0.755	0.04627916	0.48	0.98	3.47	0.29	0.009225	0.52	0.99
	145	3.2	44.5	45.0	0.49	0	833	7.61	1.46	7.89	0.08	0.839				7.43	0.03			
	265	6.3	41.9	42.3	0.38	0	833	5.80	1.69	5.80	0.00	0.775				5.64	0.02			
	265	6.3	41.7	42.1	0.40	0	341	2.39	0.85	2.51	0.01	0.738				2.79	0.16			
	580	19.2	29.8	30.2	0.40	0	341	2.88	1.64	2.39	0.24	0.638				2.96	0.01			

Summary

Model	Average k	Median k
1st order	0.01030751	0.00384
2nd Order	0.00323942	0.00055

Filtered Waters

	Reactor Data			Flow Data		NaOCl Data				1st Order Modeling Info						2ndOrder Modeling Info				
Date	Volume (mL)	t_{theo} (min)	Q_a (mL/min)	Q_d (mL/min)	Q_t (mL/min)	C_o (mg/L)	C_d (mg/L)	C_t (mg/L)	$C_{consumed}$ (mg/L)	C_{calc}	$(C_{calc}-C_t)^2$	C_tQ_t/C_dQ_d	k	SSE	r^2	C_{calc}	$(C_{calc}-C_t)^2$	k	SSE	r^2
22-Apr-00 crypto	580	31.0	18.5	0.21	18.7	0	713	6.04	1.96	7.03	0.98	0.755	0.004474	38.0	0.40	6.83	0.62	0.0008135	38.17	0.41
	580	34.4	16.6	0.24	16.8	0	436	3.94	2.27	5.38	2.09	0.634				5.40	2.12			
	580	28.1	20.4	0.25	20.7	0	248	1.88	1.12	2.67	0.62	0.626				2.82	0.88			
	580	33.4	17.1	0.25	17.4	0	248	2.03	1.54	3.11	1.16	0.588				3.28	1.56			
	1230	65.4	18.6	0.22	18.8	0	713	5.26	3.07	6.45	1.41	0.631				6.25	0.99			
	1230	74.9	16.2	0.22	16.4	0	500	10.42	-3.72	5.02	29.19	1.555				5.11	28.21			
	1230	61.7	19.7	0.24	19.9	0	500	3.27	2.75	4.72	2.09	0.543				4.84	2.47			
	2250	93.5	23.8	0.26	24.1	0	870	6.54	2.86	6.63	0.01	0.696				6.34	0.04			
	2250	87.8	25.3	0.32	25.6	0	500	4.22	2.03	4.48	0.07	0.676				4.68	0.21			
	2250	86.7	25.7	0.24	25.9	0	436	2.25	1.78	2.91	0.43	0.558				3.28	1.05			
20-Sep-00 crypto	2250	92.9	23.8	0.41	24.2	0	67400	1115.07	26.36	1080.89	1168.40	0.977	0.006603	16961.7	0.98	641.79	223990.04	0.0000131	378630.62	0.94
	2250	83.4	26.6	0.39	27.0	0	32000	473.33	-10.94	440.27	1092.73	1.024				338.05	18300.33			
	2250	84.1	26.4	0.36	26.8	0	8180	72.57	37.47	104.74	1034.75	0.659				99.24	711.15			
	1230	54.3	22.3	0.37	22.7	0	67400	960.67	139.37	1065.21	10929.17	0.873				725.39	54886.56			
	1230	56.1	21.6	0.34	21.9	0	32000	520.33	-24.43	479.69	1651.56	1.049				386.56	17894.64			
	580	28.1	20.3	0.31	20.6	0	67400	1029.38	-15.60	996.87	1056.74	1.015				786.55	58968.21			
	580	27.3	20.9	0.35	21.3	0	32000	516.00	11.06	518.53	6.40	0.979				453.72	3878.55			
	580	28.3	20.2	0.33	20.5	0	8180	124.60	6.89	129.28	21.94	0.948				125.66	1.13			
1-Aug-00 crypto	2250	83.1	26.7	0.35	27.1	0	780	4.15	5.93	2.65	2.27	0.412	0.033808	6.1	0.28	1.58	6.64	0.0412212	14.00	0.21
	2250	88.9	24.9	0.38	25.3	0	550	3.08	5.18	2.06	1.03	0.372				1.37	2.91			
	2250	89.8	24.8	0.25	25.1	0	550	1.39	4.10	1.36	0.00	0.253				1.09	0.09			
	1230	56.1	21.7	0.25	21.9	0	780	3.28	5.43	3.01	0.08	0.377				1.74	2.39			
	1230	67.2	18.0	0.33	18.3	0	360	1.90	4.49	1.95	0.00	0.297				1.35	0.30			
	580	26.8	21.4	0.23	21.7	0	780	3.51	4.77	4.35	0.70	0.424				2.32	1.40			
	580	28.0	19.7	0.27	20.0	0	550	2.56	4.87	3.75	1.42	0.344				2.11	0.20			
	580	32.0	17.8	0.29	18.1	0	360	1.97	3.69	2.72	0.57	0.347				1.73	0.06			
7-Apr-01 crypto	2250	168.8	13.2	0.13	13.3	0	4579	37.77	6.89	35.56	4.89	0.846	0.001516	329.190	0.01	40.60	8.01	0.0000146	441.98	0.92
	2250	173.9	12.8	0.14	12.9	0	7977	63.77	19.48	65.88	4.46	0.766				70.61	46.80			
	2250	187.2	11.9	0.12	12.0	0	12408	100.93	22.94	96.49	19.70	0.815				97.78	9.91			
	1230	116.0	10.5	0.10	10.6	0	4579	37.18	6.02	36.74	0.20	0.861				40.43	10.58			
	1230	106.0	11.5	0.10	11.6	0	7977	58.1	10.67	59.24	1.31	0.845				62.69	21.09			
	1230	108.8	11.2	0.10	11.3	0	12408	94.73	15.08	94.25	0.23	0.863				95.37	0.41			
	580	51.3	11.2	0.10	11.3	0	4579	42.18	-1.66	37.60	21.01	1.041				39.36	7.94			
	580	56.3	10.2	0.10	10.3	0	7977	54.7	22.75	71.36	277.40	0.706				73.06	337.25			
24-Mar-01	145	3.6	40.0	0.47	40.5	0	341	2.85	1.11	3.48	0.40	0.720	0.0385	1.463	0.94	3.61	0.58	0.0075	0.95	0.96
	145	3.2	45.2	0.49	45.7	0	833	7.98	0.95	7.96	0.00	0.893				7.57	0.17			
	265	6.3	41.4	0.40	41.8	0	833	5.82	2.15	6.41	0.35	0.730				6.16	0.12			
	265	6.2	42.1	0.36	42.5	0	341	2.58	0.31	2.33	0.06	0.892				2.58	0.00			
	580	18.5	31.0	0.40	31.4	0	341	3.35	0.99	2.54	0.66	0.771				3.05	0.09			

Summary	Model	Average k	Median k
	1st order	0.010099996	0.00299
	2nd Order	0.010051557	0.00041

Appendix I.3
Residual Chlorine Determination at Low Detention Time
Conducted: 24 March 2001

	First-order decay	
	k =	0.0463
	C_{F-calc}	$(C_F - C_{F-calc})^2$
	3.28	0.12
	7.90	0.09
	5.81	0.00
	2.39	0.24
	2.51	0.01
SUM		0.46
STDEV	2.40	
r		0.991
r^2		0.983

	Second-order decay	
	k =	0.0092
	C_{F-calc}	$(C_F - C_{F-calc})^2$
	3.44	0.26
	7.44	0.03
	5.65	0.02
	2.96	0.01
	2.79	0.16
SUM		0.47
STDEV	2.02	
r		0.997
r^2		0.993

Reactor	V	t_{nom}	Q_o	Q_D	Q_F	C_o	C_D	C_F	C_fQ_f/C_dQ_d
R1	0.145	3.7	39.1	0.45	39.5	0	341	2.93	0.76
R2	0.145	3.2	44.5	0.49	44.9	0	833	7.61	0.84
R3	0.265	6.3	41.9	0.38	42.2	0	833	5.80	0.77
R5	0.58	19.2	29.8	0.40	30.2	0	341	2.88	0.64
R7	0.265	6.3	41.7	0.40	42.1	0	341	2.39	0.74

t_{nom} (min)	1st order
0	1.0000
5	0.8122
10	0.6837
15	0.5904
20	0.5194
25	0.4637
30	0.4188
35	0.3818
40	0.3508
45	0.3245
50	0.3019
55	0.2822
60	0.2649
65	0.2496
70	0.2360
75	0.2237
80	0.2127
85	0.2028
90	0.1937
95	0.1854
100	0.1778
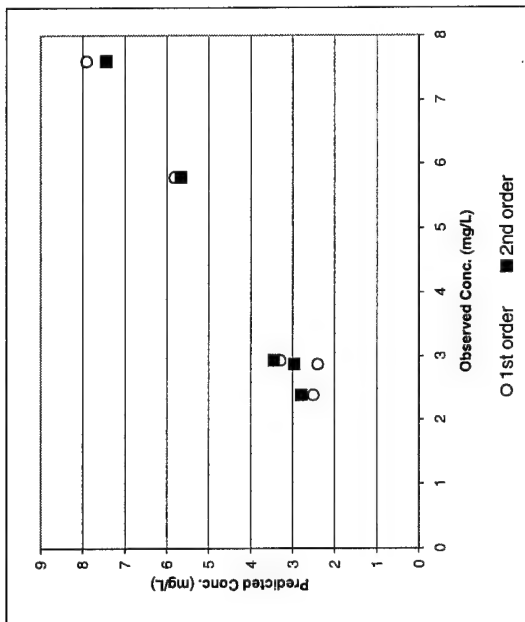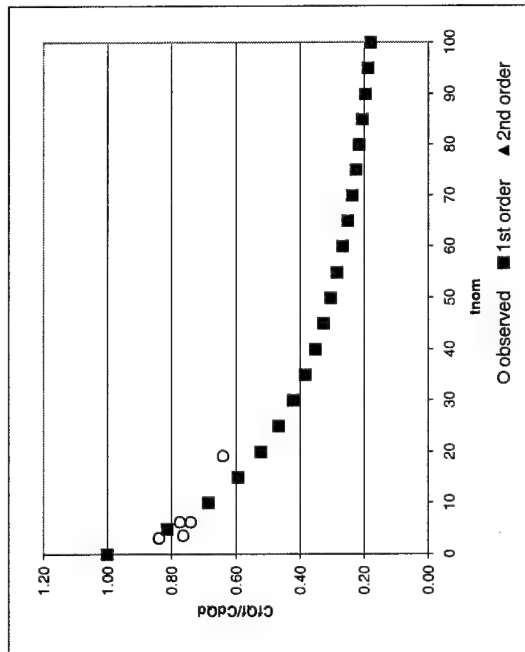
Appendix I.4
Residual Chlorine Determination at Low Detention Time
Conducted: 24 March 2001

Reactor	V	t_{nom}	Q_0	Q_D	Q_F	C_0	C_D	C_F	CfQf/CdQd	First-order decay k = 0.0385		Second-order decay k = 0.0075	
										C_{F-calc}	$(C_F - C_{F-calc})^2$	C_{F-calc}	$(C_F - C_{F-calc})^2$
R1	0.145	3.6	40.0	0.47	40.5	0	341	2.85	0.72	3.48	0.40	3.61	0.58
R2	0.145	3.2	45.2	0.49	45.7	0	833	7.98	0.89	7.96	0.00	7.57	0.16
R3	0.265	6.3	41.4	0.40	41.8	0	833	5.82	0.73	6.40	0.34	6.17	0.12
R5	0.58	18.5	31.0	0.40	31.4	0	341	3.35	0.77	2.54	0.67	3.06	0.09
R7	0.265	6.2	42.1	0.36	42.4	0	341	2.58	0.90	2.30	0.08	2.55	0.00
									SUM		1.48	SUM	0.95
									STDEV		2.52	STDEV	2.17
									r		0.972	r	0.979
									r^2		0.944	r^2	0.959

t_{nom} (min)	1st order
0	1.0000
5	0.8384
10	0.7218
15	0.6336
20	0.5647
25	0.5092
30	0.4637
35	0.4257
40	0.3934
45	0.3657
50	0.3416
55	0.3205
60	0.3018
65	0.2852
70	0.2704
75	0.2570
80	0.2449
85	0.2338
90	0.2237
95	0.2145
100	0.2060
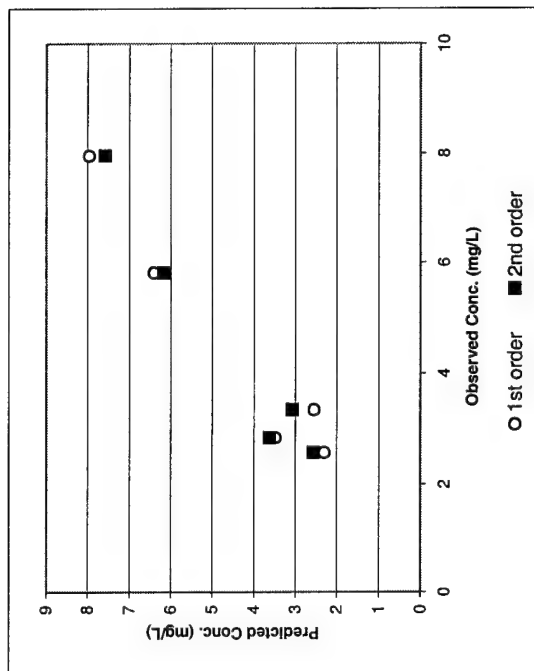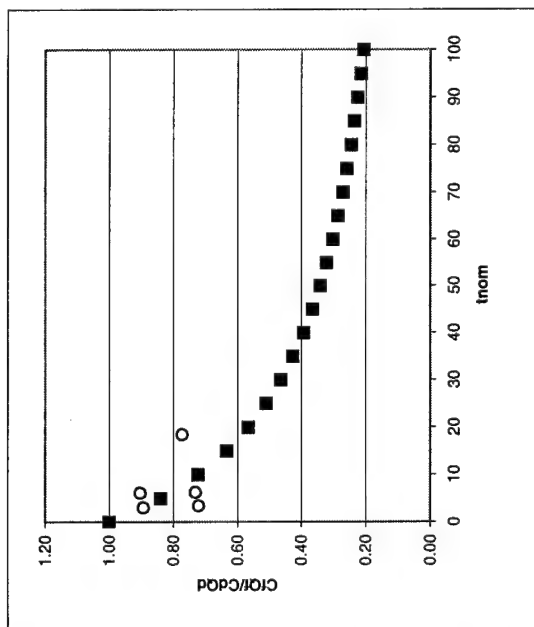
Appendix I.5
Chlorine Decay
Batch Reactor Analysis

Determination of the Order of Reaction (2nd order overall; 1st WRT A and B)

2nd overall; 1st wrt A, B

pH 6 Time (min)	Raw Water (mg/L)	$C_o =$ 2.4008 $K =$ 0.0089 $a =$ 0		$C_o =$ 2.5580 $K =$ 0.0062 $a =$ 1		$C_o =$ 2.7762 $K =$ 0.0041 $a =$ 2		$C_{a0} =$ 3.2808 $C_{b0} =$ 1.8451 $k =$ 0.0235	
		Raw Water							
		Theoretical	obs-theo	Theoretical	obs-theo	Theoretical	obs-theo	Theoretical	obs-theo
1	3.1	2.3918	0.5015	2.5424	0.3110	2.7448	0.1261	3.1465	0.0022
9	2.53	2.3205	0.0439	2.4203	0.0120	2.5172	0.0002	2.4540	0.0058
13	2.35	2.2849	0.0042	2.3614	0.0001	2.4170	0.0045	2.2521	0.0096
25	1.86	2.1779	0.1010	2.1934	0.1111	2.1591	0.0894	1.8933	0.0011
40	1.42	2.0442	0.3896	2.0000	0.3364	1.9050	0.2352	1.6804	0.0678
55	1.60	1.9104	0.0964	1.8237	0.0500	1.7044	0.0109	1.5738	0.0007
80	1.60	1.6876	0.0077	1.5637	0.0013	1.4499	0.0225	1.4920	0.0117
100	1.51	1.5093	0.0000	1.3827	0.0162	1.2952	0.0461	1.4638	0.0021
150	1.42	1.0635	0.1271	1.0165	0.1628	1.0225	0.1580	1.4408	0.0004
		Sum =	1.2714	Sum =	1.0010	Sum =	0.6929	Sum =	0.1014
		r =	0.7415	r =	0.8065	r =	0.8775	r =	0.9819
		$r^2 =$	0.5499	$r^2 =$	0.6504	$r^2 =$	0.7700	$r^2 =$	0.9641

2nd overall; 1st wrt A, B

pH 6 Time (min)	Treated Water (mg/L)	$C_o =$ 2.8928 $K =$ 0.0121 $a =$ 0		$C_o =$ 2.9876 $K =$ 0.0063 $a =$ 1		$C_o =$ 3.0786 $K =$ 0.0031 $a =$ 2		$C_{a0} =$ 3.0327 $C_{b0} =$ 5.4054 $k =$ 0.0014	
		Raw Water							
		Theoretical	obs-theo	Theoretical	obs-theo	Theoretical	obs-theo	Theoretical	obs-theo
1	3.10	2.8808	0.0481	2.9687	0.0172	3.0491	0.0026	3.0091	0.0083
5	3.01	2.8326	0.0315	2.8943	0.0134	2.9364	0.0054	2.9175	0.0086
10	2.66	2.7723	0.0126	2.8039	0.0207	2.8067	0.0215	2.8089	0.0222
20	2.44	2.6518	0.0449	2.6314	0.0366	2.5789	0.0193	2.6096	0.0288
36	2.39	2.4590	0.0048	2.3773	0.0002	2.2825	0.0116	2.3326	0.0033
58	2.13	2.1939	0.0041	2.0675	0.0039	1.9710	0.0253	2.0182	0.0125
123	1.37	1.4106	0.0016	1.3686	0.0000	1.4046	0.0012	1.3792	0.0001
153	1.15	1.0491	0.0102	1.1313	0.0003	1.2402	0.0081	1.1770	0.0007
		Sum =	0.1577	Sum =	0.0924	Sum =	0.0950	Sum =	0.0844
		r =	0.9773	r =	0.9868	r =	0.9865	r =	0.9879
		$r^2 =$	0.9551	$r^2 =$	0.9737	$r^2 =$	0.9732	$r^2 =$	0.9760

Notes:
1. A Batch Reactor Chlorine Analysis was conducted to evaluate the disinfectant demand from 0 to 3 minutes.
2. This data will be used to model future experiments for CMFTR analysis of the disinfectant demand.
3. Procedure:
   a. Collect water samples. Let stand until Temperature = 20C.
   b. Adjust to pH 6.
   c. Add 20 mL of 500 mg/L NaOCl stock solution to 2 L of sample water.
   d. Mix for I minute.
   e. Sample for chlorine residual.
   f. Distribute contents of solution into 250 mL reactors with zero headspace and stir until sampled.
   g. Sample chlorine residual over time.
4. Once this data was collected, models for chlorine decay in batch reactors was used to approximate best fits of kd.

Appendix I.6
Chlorine Decay
CMFTR Model Predictions

Batch Analysis Data		Simple 1st Order		Simple 2nd Order	2nd Order Overall
pH 6	Raw Water	C_{e0} = 3.281		C_{e0} = 3.281	C_{e0} = 3.281
		k_d = 0.0062		k_d = 0.0041	C_{b0} = 1.8451
					k_d = 0.0235
Time (min)	(mg/L)	Time (min)	C_r (mg/L)	C_r (mg/L)	C_r (mg/L)
1	3.1	1	3.26	3.24	3.15
9	2.53	9	3.11	2.96	2.62
13	2.35	13	3.04	2.85	2.48
25	1.86	25	2.84	2.59	2.23
40	1.42	40	2.63	2.36	2.06
55	1.60	55	2.45	2.19	1.96
80	1.60	80	2.20	1.98	1.85
100	1.51	100	2.03	1.86	1.79
150	1.42	150	1.71	1.63	1.70

Batch Analysis Data		Simple 1st Order		Simple 2nd Order	2nd Order Overall
pH 6	Treated Water	C_{e0} = 3.033		C_{e0} = 3.033	C_{e0} = 3.033
		k_d = 0.0063		k_d = 0.0031	C_{b0} = 5.4054
					k_d = 0.0014
Time (min)	(mg/L)	Time (min)	C_r (mg/L)	C_r (mg/L)	C_r (mg/L)
1	3.10	1	3.01	3.00	3.01
5	3.01	5	2.94	2.90	2.92
10	2.66	10	2.85	2.79	2.82
20	2.44	20	2.69	2.61	2.65
36	2.39	36	2.47	2.39	2.43
58	2.13	58	2.22	2.17	2.19
123	1.37	123	1.70	1.79	1.75
153	1.15	153	1.54	1.68	1.61

CMFTR Chlorine Decay Prediction
Raw Water

CMFTR Chlorine Decay Prediction
Filtered Water

Analytical and Sampling Error

Experiment	Date	CF1 (oocysts/L)	CF2 (oocysts/L)	CF3 (oocysts/L)	Variance (oocysts/L)	CF1 (live oocysts/L)	CF2 (live oocysts/L)	CF3 (live oocysts/L)	Variance (live oocysts/L)
1	3-Apr-00	5.419	5.524	5.381	0.005	5.414	5.511	5.372	0.005
2	22-Apr-00	6.392	6.548	6.420	0.007	6.328	6.456	6.369	0.004
3	7-May-00	5.774	5.704	5.745	0.001	5.751	5.673	5.710	0.002
6	1-Aug-00	6.097	6.131	6.182	0.002	6.048	6.072	6.096	0.001
7	12-Sep-00	5.900	5.828	6.065	0.015	5.849	5.775	6.022	0.016
8	20-Sep-00	5.896	5.957	5.940	0.001	5.878	5.940	5.921	0.001
9	26-Sep-00	6.191	6.081	6.027	0.007	6.155	6.042	5.996	0.007
11	24-Feb-01	6.050	6.158	6.229	0.008	6.030	6.133	6.205	0.008
13	7-Apr-01	6.031	6.031	6.007	0.000	5.984	5.974	5.950	0.000
				Average	0.005			Average	0.005
				Std Dev	0.072			Std Dev	0.069

Experimental Error

Experiment	Date	CF1 (oocysts/L)	CF2 (oocysts/L)	CF3 (oocysts/L)	Variance (oocysts/L)	Standard Deviation
1	3-Apr-00	5.419	5.524	5.381	0.005	0.074
7	12-Sep-00	5.900	5.828	6.065	0.015	0.122

Experiment	Date	Reactor (L)	1 (oocysts/L)	2 (oocysts/L)	3 (oocysts/L)	4 (oocysts/L)	Variance (oocysts/L)
1	3-Apr-00	2.250	6.026	5.475	5.730	5.261	0.109
		1.230	5.137	6.063	5.483	NA	0.219
		0.580	5.747	6.019	5.673	NA	0.033
						Average	0.120
						Std Dev	0.347

Experiment	Date	Reactor (L)	1 (oocysts/L)	2 (oocysts/L)	3 (oocysts/L)	Variance (oocysts/L)
7	12-Sep-00	2.250	6.173	5.990	6.378	0.038
		1.230	6.054	6.447	6.112	0.045
		0.580	6.203	6.095	6.028	0.008
					Average	0.030
					Std Dev	0.174

Experiment	Date	CF1 (oocysts/L)	CF2 (oocysts/L)	Variance (oocysts/L)	CF1 (live oocysts/L)	CF2 (live oocysts/L)	Variance (live oocysts/L)
6	1-Aug-00	6.652	6.689	0.001	5.569	5.508	0.002
9	26-Sep-00	7.415	7.275	0.010	8.004	7.851	0.012
11	24-Feb-01	7.998	8.202	0.021	8.569	8.804	0.028
13	7-Apr-01	7.365	7.270	0.005	7.953	7.852	0.005
			Average	0.009		Average	0.012
			Std Dev	0.095		Std Dev	0.108

Appendix K
Flow Cytometry Data

NSF Experiment 10 -2/24/01

Sample name	Total volume	Flowcount Crypto	Crypto/ul	Total Crypto	% SYTO positive	Comments
feed 1	695	770	80.78	56,144.41	4.6	
feed 2	675	1016	106.57	71,935.13	5.5	
feed 3	590	1369	143.63	84,739.61	5.5	
ctrl 1	535	86594	9,084.87	4,860,406.48	4.6	
ctrl 2	450	168822	17,676.10	7,954,243.95	0.0	
r1	650	1263	132.32	86,007.43	45.3	
r2	485	1657	174.26	84,517.37	52.8	
r3	530	2097	219.69	`116,437.69	46.5	
r5	450	1759	184.21	82,893.98	41.4	
r6	495	1182	123.83	61,297.38	30.1	
r7	420	3689	387.42	162,714.57	75.8	
r9	450	2485	260.19	117,083.65	35.0	
r10	630	1507	158.17	99,645.97	27.1	
r11	730	1693	177.30	129,426.87	33.7	
raw	480	40	4.20	2,014.34		
raw	490	13	1.36	668.70		
raw	530	13	1.36	721.98		
live					1.8	
heat inactivated control					82.9	

NSF Experiment 13 -4/07/01

Sample name	Total volume	Flowcount Crypto	Crypto/ul	Total Crypto	% SYTO positive	Comments
feed 1	470	1091	114.25	53,699.13	10.2	
feed 2	490	1048	109.68	53,745.26	12.4	
feed 3	415	1169	122.30	50,754.08	12.2	
ctrl 1	355	31086	3,261.34	1,157,775.10	3.1	
ctrl 2	330	25569	2,823.54	931,767.31	4.5	
r1	300	3350	351.67	163,448.92	23.9	
r2	300	1090	144.54	43,361.42	15.8	
r3	140	445	117.57	16,459.58	21.0	40 ul for enumeration
r5	565	1284	67.92	38,372.05	31.3	200 ul for enumeration
r6	365	729	444.22	162,140.68	30.2	
r7	345	621	66.22	22,845.20	26.1	
r9	750	1071	392.40	294,299.05	66.9	200 ul for enumeration
r10	260	1123	292.83	76,136.93	66.8	low numbers for viability determination
filtered h2o	400	42	4.40	1,761.13	nd	
live					1.8	
heat inactivated control					97.2	

Note:
Total Volume = uL after centrifuge to concentrate crypto
Flowcount crypto is the total number crypto in centrifuged volume
Crypto/uL is corrected concentration based upon flow count beads
Total Crypto = crypto/uL x total volume and is the #oocysts in 50-mL
%PI positive = % dead
% SYTO positive = % dead

FEED TANK
Added 3E+08 oocysts to 145 L raw water.

Feed Volume =	145	L
Total oocysts =	3.0E+08	oocysts
Conc. of oocysts =	2.1E+06	oocysts/L
log Conc. =	6.3	oocysts/L

CONTROL
Added 3E+06 oocysts to 200 mL DI water.

Feed Volume =	200	mL
Total oocysts =	3.0E+06	oocysts
Conc. of oocysts =	1.5E+07	oocysts/L
log Conc. =	7.2	oocysts/L

CONTROL SAMPLES

Sample	Total volume (µL)	Flowcount Crypto (oocysts)	Crypto/µl (oocysts/µL)	Total Crypto (oocysts)	% SYTO positive	Total Crypto (oocysts)	Crypto Conc (oocysts/L)	Log Conc (oocysts/L)	Live Crypto (oocysts)	Live Crypto Conc (oocysts/L)	Log Live Conc (oocysts/L)
CTRL1	535	86594	9084.87193	4860406.482	4.6	1.94E+07	9.72E+07	7.988	1.85E+07	3.71E+08	8.569
CTRL2	450	168822	17676.0977	7954243.951	0	3.18E+07	1.59E+08	8.202	3.18E+07	6.36E+08	8.804
					SUM =	5.13E+07	2.56E+08	16.189	5.04E+07	1.01E+09	17.373
					AVG =	2.56E+07	1.28E+08	8.095	2.52E+07	5.04E+08	8.686
					STD DEV =	8.75E+06	4.38E+07	0.151	9.38E+06	1.88E+08	0.166

FEED TANK SAMPLES

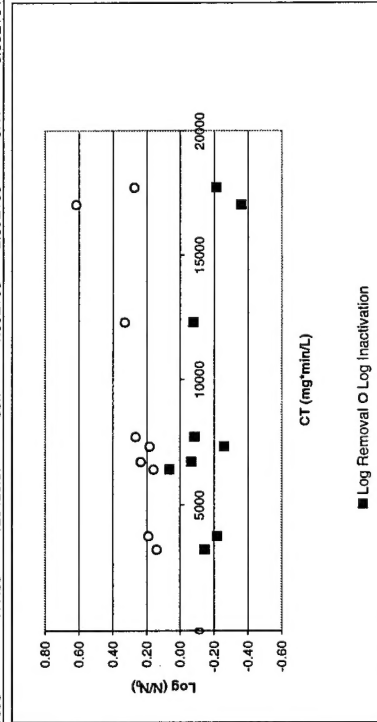| Sample	Total volume (µL)	Flowcount Crypto (oocysts)	Crypto/µl (oocysts/µL)	Total Crypto (oocysts)	% SYTO positive	Total Crypto (oocysts)	Crypto Conc (oocysts/L)	Log Conc (oocysts/L)	Live Crypto (oocysts)	Live Crypto Conc (oocysts/L)	Log Live Conc (oocysts/L)
CF1	695	770	80.7833266	56144.412	4.6	1.63E+08	1.12E-06	6.050	5.36E+04	1.07E+06	6.030
CF2	675	1016	106.570566	71935.13187	5.5	2.09E+08	1.44E+06	6.158	6.80E+04	1.36E+06	6.133
CF3	590	1369	143.62646	84739.61136	5.5	2.46E+08	1.69E+06	6.229	8.01E+04	1.60E+06	6.205
					SUM =	6.2E+08	4.3E-06	18.44	2.0E+05	4.0E+06	18.37
					AVG =	2.1E+08	1.4E-06	6.15	6.7E+04	1.3E+06	6.12
					STD DEV =	4.E+07	286471	0.09	13275	265509	0.09

REACTOR SAMPLES

Sample	Total volume (µL)	Flowcount Crypto (oocysts)	Crypto/µl (oocysts/µL)	Total Crypto (oocysts)	% SYTO positive	Total Crypto (oocysts)	Crypto Conc (oocysts/L)	Log Conc (oocysts/L)	Live Crypto (oocysts)	Live Crypto Conc (oocysts/L)	Log Live Conc (oocysts/L)	Cl₂ Conc (mg/L)	T_nom (min)	CT (min*mg/L)	Log Removal	Log Inactivation
R1	650	1263	132.32	86007.43	45.3	3.87E+06	1.72E+06	6.24	4.70E+04	9.41E-05	5.97	48.83	158.7	7748	-0.08	0.26
R2	485	1657	174.26	84517.37	52.8	3.80E+06	1.69E+06	6.23	3.99E+04	7.98E-05	5.90	74.74	164.8	12315	-0.08	0.33
R3	530	2097	219.69	116437.69	46.5	5.24E+06	2.33E+06	6.37	6.23E+04	1.25E-06	6.10	110.77	160.0	17720	-0.22	0.27
R5	450	1759	184.21	82893.98	41.4	2.04E+06	1.66E+06	6.22	4.86E+04	9.72E-05	5.99	60.33	111.6	6735	-0.07	0.23
R6	495	1182	123.83	61297.38	30.1	1.51E+06	1.23E+06	6.09	4.28E+04	8.57E-05	5.93	68.50	93.8	6427	0.06	0.16
R7	420	3689	387.42	162714.57	75.8	4.00E+06	3.25E+06	6.51	3.94E+04	7.88E-05	5.90	140.62	121.1	17028	-0.36	0.62
R9	450	2485	260.19	117083.65	35	1.36E+06	2.34E+06	6.37	7.61E+04	1.52E-06	6.18	59.99	62.8	3768	-0.22	0.19
R10	630	1507	158.17	99645.97	27.1	1.16E+06	1.99E+06	6.30	7.26E+04	1.45E-06	6.16	67.47	47.9	3234	-0.15	0.14
R11	730	1693	177.30	129426.87	33.7	1.50E+06	2.59E+06	6.41	8.58E+04	1.72E-06	6.23	134.61	54.7	7359	-0.26	0.18
													AVG		-0.17	0.26
													STDEV		0.14	0.15

CT (mg*min/L)

■ Log Removal ○ Log Inactivation

Appendix L.2
RUN #13
Conducted: 7 April 2001
Log Inactivation and Removal Analysis

FEED TANK
Added 3E+08 oocysts to 145 L raw water.

Feed Volume =	135	L
Total oocysts =	1.8E+08	oocysts
Conc. of oocysts =	1.3E+06	oocysts/L
log Conc. =	6.1	oocysts/L

CONTROL
Added 3E+06 oocysts to 200 mL DI water.

Feed Volume =	200	mL
Total oocysts =	1.8E+06	oocysts
Conc. of oocysts =	9.0E+06	oocysts/L
log Conc. =	7.0	oocysts/L

CONTROL SAMPLES

Sample	Total volume (µL)	Flowcount Crypto (oocysts)	Crypto/µL (oocysts/µL)	Total Crypto (oocysts)	Crypto Conc (oocysts/L)	Log Conc (oocysts/L)	% SYTO positive	Total Crypto (oocysts)	Live Crypto (oocysts)	Live Crypto Conc (oocysts/L)	Log Live Conc (oocysts/L)
ctrl 1	355	31086	3,261.34	1,157,775.10	2.32E+07	7.365	3.1	4.63E+06	4.49E+06	8.98E+07	7.953
ctrl 2	330	25569	2,823.54	931,767.31	1.86E+07	7.270	4.5	3.73E+06	3.56E+06	7.12E+07	7.852
SUM =								8.36E+06		1.61E+08	15.805
AVG =								4.18E+06		8.05E+07	7.903
STD DEV =								3.20E+06		1.31E+07	0.071

FEED TANK SAMPLES

| Sample	Total volume (µL)	Flowcount Crypto (oocysts)	Crypto/µL (oocysts/µL)	Total Crypto (oocysts)	Total Crypto (oocysts)	Crypto Conc (oocysts/L)	Log Conc (oocysts/L)	% SYTO positive	Total Crypto (oocysts)	Live Crypto (oocysts)	Live Crypto Conc (oocysts/L)	Log Live Conc (oocysts/L)
feed 1	470	1091	114.25	53,699.13	1.45E+08	1.07E+06	6.031	10.2	1.45E+08	4.82E+04	9.64E+05	5.984
feed 2	490	1048	109.68	53,745.26	1.45E+08	1.07E+06	6.031	12.4	1.45E+08	4.71E+04	9.42E+05	5.974
feed 3	415	1169	122.30	50,754.08	1.37E+08	1.02E+06	6.007	12.2	1.37E+08	4.46E+04	8.91E+05	5.950
SUM =					4.3E+08		18.069		1.4E+05		2.8E+06	17.908
AVG =					1.4E+08		6.023		4.7E+04		9.3E+05	5.969
STD DEV =					5.E+06		0.014		1873		37452	0.018

REACTOR SAMPLES

Sample	Total volume (µL)	Flowcount Crypto (oocysts)	Crypto/µL (oocysts/µL)	Total Crypto (oocysts)	Crypto Conc (oocysts/L)	Log Conc (oocysts/L)	% SYTO positive	Total Crypto (oocysts)	Live Crypto (oocysts)	Live Crypto Conc (oocysts/L)	Log Live Conc (oocysts/L)	Cl_2 Conc (mg/L)	T_{nom} (min)	CT (min*mg/L)	Log Removal	Log Inactivation
r1	300	3350	351.67	105501.71	2.11E+06	6.324	23.9	4.75E+06	8.03E+04	1.61E+06	6.206	47.50	168.7	8013	-0.301	0.119
r2	300	1090	144.54	43361.42	8.67E+05	5.938	15.8	1.95E+06	3.65E+04	7.30E+05	5.863	83.27	174.0	14487	0.085	0.075
r3	140	445	117.57	16459.58	3.29E+05	5.517	21	7.41E+05	1.30E+04	2.60E+05	5.415	123.70	186.9	23123	0.506	0.102
r5	565	1284	67.92	38372.05	7.67E+05	5.885	31.3	9.44E+06	2.64E+04	5.27E+05	5.722	45.98	116.0	5336	0.138	0.163
r6	365	729	444.22	162140.68	3.24E+06	6.511	30.2	3.99E+06	1.13E+05	2.26E+06	6.355	68.77	106.0	7292	-0.488	0.156
r7	345	621	66.22	22845.20	4.57E+05	5.660	26.1	5.62E+05	1.69E+04	3.38E+05	5.528	109.72	108.8	11935	0.363	0.131
r9	750	1071	392.40	294299.05	5.89E+06	6.770	66.9	3.41E+06	9.74E+04	1.95E+06	6.290	40.96	51.3	2102	-0.747	0.480
r10	260	1123	292.83	76136.93	1.52E+06	6.183	66.8	8.83E+05	2.53E+04	5.06E+05	5.704	77.38	56.3	4354	-0.160	0.479
													AVG		-0.065	0.252
													STDEV		0.490	0.178

Chart — y-axis: Log (N/N₀), values 0.600, 0.400, 0.200, 0.000, -0.200, -0.400, -0.600, -0.800, -1.000; x-axis: CT (mg*min/L), values 5000, 10000, 15000, 20000, 25000.
Legend: ■ Log Removal   ○ Log Inactivation
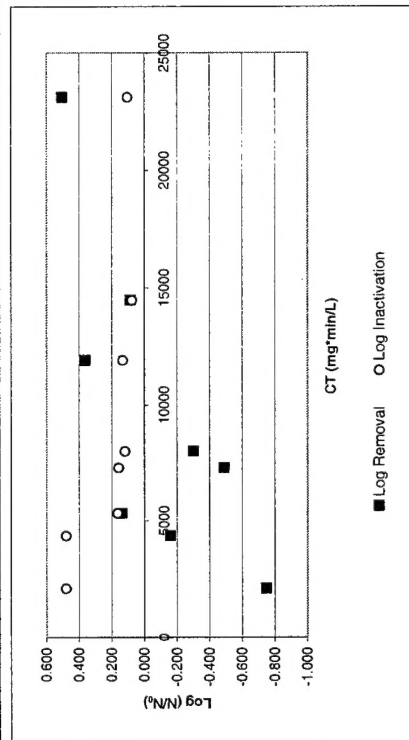
Experiment	2					
Date	22-Apr-00					
	Filtered Water					

Reactor Volume (mL)	Cl$_2$ Conc (mg/L)	τ_{nom} (min)	CT (min*mg/L)	Log Removal	Log Inactivation
2250	6.5	93.7	609	0.07	0.06
2250	4.2	87.8	369	-0.09	0.09
2250	2.3	86.7	199	0.04	0.05
1230	5.3	65.3	346	0.09	0.33
1230	10.4	74.9	779	0.11	0.19
1230	3.3	61.7	204	0.05	0.2
580	6.0	31.0	186	0.17	0.27
580	3.9	34.5	135	-0.03	0.28
580	1.9	28.1	53	-0.14	0.21
580	2.0	33.4	67	-0.25	0.27
			AVG	0.00	0.20
			Median	0.05	0.21
			STDEV	0.13	0.10

Log Inactivation verses CT
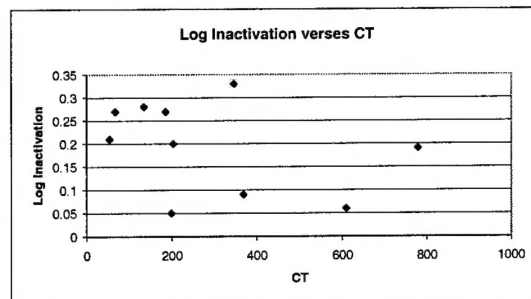
Experiment	3					
Date	7-May-00					
	Raw Water					

Reactor Volume (mL)	Cl$_2$ Conc (mg/L)	τ_{nom} (min)	CT (min*mg/L)	Log Removal	Log Inactivation
2250	5.7	81.3	459	-0.03	0.02
2250	7.2	87.7	633	0.04	0.03
2250	5.0	89.9	451	0	0.04
1230	2.5	48.5	121	0.28	0.05
1230	6.8	60.4	411	0.13	0.07
1230	4.1	65.4	266	0.23	0.05
1230	1.8	57.8	103	0.27	0.07
			AVG	0.13	0.05
			Median	0.13	0.05
			STDEV	0.13	0.02

Log Inactivation verses CT
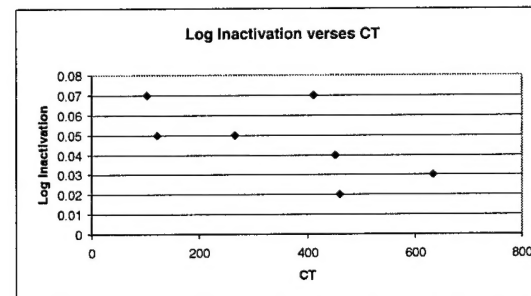
Experiment	6					
Date	1-Aug-00					
	Raw Water					

Reactor Volume (mL)	Cl$_2$ Conc (mg/L)	τ_{nom} (min)	CT (min*mg/L)	Log Removal	Log Inactivation
2250	4.2	83.1	345	0.06	0.07
2250	3.1	88.9	274	0.02	0.09
2250	1.4	89.8	125	0.04	0.10
1230	3.3	56.1	184	0.04	0.06
1230	2.8	59.8	167	-0.04	0.07
1230	1.9	67.2	128	0.06	0.09
580	3.5	26.8	94	0.13	0.06
580	2.6	29.0	74	0.07	0.07
580	2.0	32.0	63	0.37	0.05
			AVG	0.08	0.07
			Median	0.06	0.07
			STDEV	0.12	0.02
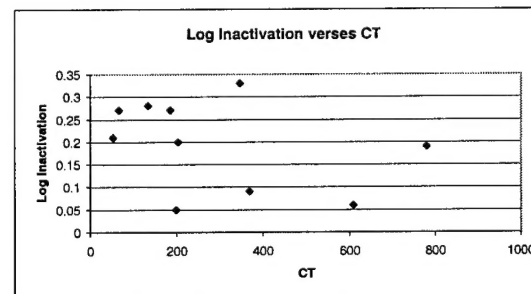
Log Inactivation verses CT

Experiment	8				
Date	20-Sep-00				
	Filtered Water				

Reactor Volume (mL)	Cl₂ Conc (mg/L)	τ_nom (min)	CT (min*mg/L)	Log Removal	Log Inactivation
2250	473.3	83.5	39521	0.16	0.25
2250	72.6	84.2	6113	0.16	0.25
580	516.0	27.3	14087	0.3	0.37
580	124.6	28.3	3526	0.35	0.43
			AVG	0.24	0.33
			Median	0.23	0.31
			STDEV	0.10	0.09

Log Inactivation verses CT
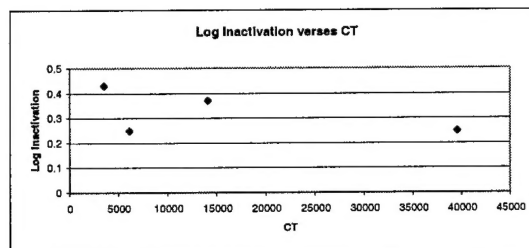
Experiment	9				
Date	26-Sep-00				
	Raw Water				

Reactor Volume (mL)	Cl₂ Conc (mg/L)	τ_nom (min)	CT (min*mg/L)	Log Removal	Log Inactivation
2250	141.5	87.0	12311	0.05	0.07
1230	130.2	57.7	7513	0.06	0.06
580	578.7	28.2	16319	0.03	0.04
580	127.4	29.6	3771	0.05	0.04
			AVG	0.05	0.05
			Median	0.05	0.05
			STDEV	0.01	0.02

Log Inactivation verses CT
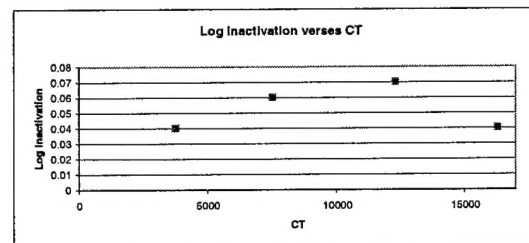
Experiment	11				
Date	24-Feb-01				
	Raw Water				

Reactor Volume (mL)	Cl₂ Conc (mg/L)	τ_nom (min)	CT (min*mg/L)	Log Removal	Log Inactivation
2250	48.9	158.7	7760	-0.08	0.26
2250	74.7	164.8	12311	-0.08	0.33
2250	110.8	160.0	17728	-0.22	0.27
1230	60.3	111.6	6729	-0.07	0.23
1230	68.5	93.8	6425	0.06	0.16
1230	140.6	121.1	17027	-0.36	0.62
580	60.0	62.8	3768	-0.22	0.19
580	67.5	47.9	3233	-0.15	0.14
580	134.6	54.7	7363	-0.26	0.18
			AVG	-0.15	0.26
			Median	-0.15	0.23
			STDEV	0.13	0.15

Log Inactivation verses CT
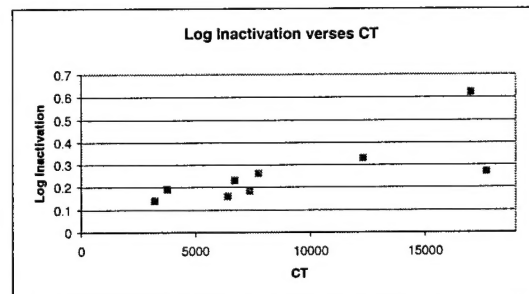
Experiment	13				
Date	7-Apr-01				
	Filtered Water				

Reactor Volume (mL)	Cl₂ Conc (mg/L)	τ_nom (min)	CT (min*mg/L)	Log Removal	Log Inactivation
2250	47.5	168.7	8013	-0.3	0.12
2250	83.3	174.0	14494	0.09	0.08
2250	123.7	186.9	23120	0.51	0.10
1230	46.0	116.0	5336	0.14	0.16
1230	68.8	106.0	7293	-0.49	0.16
1230	109.7	108.8	11935	0.36	0.13
580	41.0	51.3	2103	-0.75	0.48
580	77.4	56.3	4358	-0.16	0.48
			AVG	-0.08	0.21
			Median	-0.04	0.15
			STDEV	0.43	0.17

Log Inactivation verses CT